

**A STUDY OF CHEMICAL CONSTITUENTS OF  
CERTAIN MEDICINAL PLANTS OF  
BUNDELKHAND REGION**

**A THESIS ( SUMMARY )  
SUBMITTED FOR THE DEGREE OF  
Doctor Of Philosophy  
OF  
BUNDELKHAND UNIVERSITY**

By  
*Km Madhu Vashneya*  
M.Sc.



**CHEMICAL LABORATORY  
DAYANAND VEDIC (P.G.) COLLEGE  
ORAI-285001  
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## SUMMARY

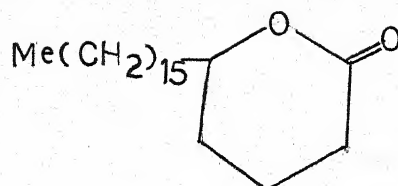
The present thesis entitled, 'A STUDY OF CHEMICAL CONSTITUENTS OF CERTAIN MEDICINAL PLANTS OF BUNDELKHAND REGION' deals with the isolation and chemical examination of some chemical constituents from the leaves of *Butea monosperma*, the rhizome of *Curcuma longa* and bark of *Ficus glomerata*. The whole work has been presented in the form of four chapters.

The first chapter is introductory which mainly consists of a brief description of the nature of different constituents such as terpenoids, flavanoids (flavanones and aurone), lactones and essential oils.

The second chapter deals with the survey of literature, isolation, purification and structural studies of four compounds (A), (B), (C) and (D) from the leaves of *Butea monosperma*.

The compound (A) was isolated with ethyl alcohol from the leaves of *Butea monosperma* and was recrystallised from ethanol: Chloroform (1:1 v/v), a white flakes having molecular formula  $C_{21}H_{40}O_2$ , m.p.  $70-71^{\circ}C$  and gave various reactions which showed the presence of  $\delta$ -lactone of Heneicosanoic acid.

Finally the structure of compound (A) was further confirmed by spectral measurements. Thus the structure of compound (A),  $\delta$ -lactone of Heneicosanoic acid has been assigned as follows.



$\delta$  - lactone of Heneicosanoic acid.



The compound (B) was isolated with ethanol from the leaves of *Butea monosperma* and its purity was checked by paper chromatography.

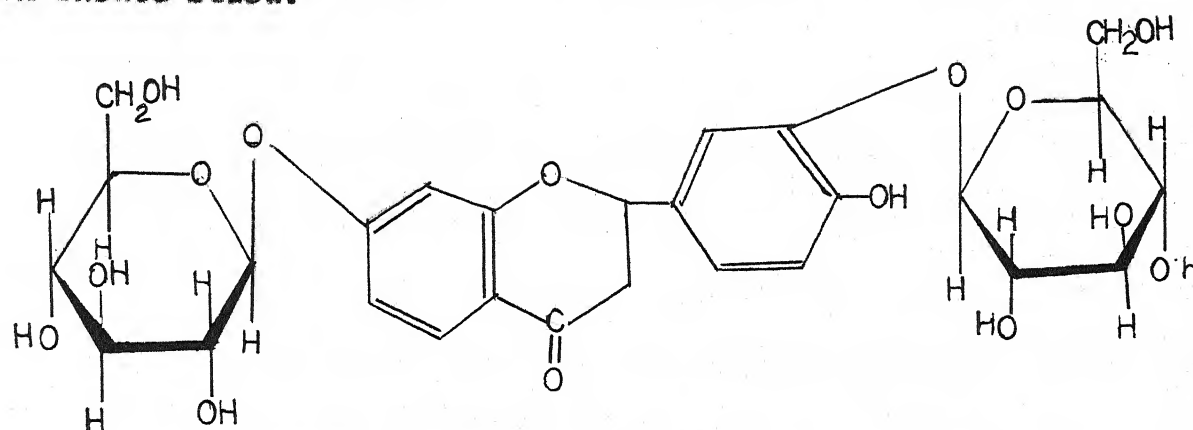
The compound (B), having molecular formula  $C_{27}H_{32}O_{15} \cdot 2H_2O$ , m.p.  $190^{\circ}C$  gave all the reactions of flavanone glycoside.

The compound (B), on acid hydrolysis with 5% dilute sulphuric acid gave an aglycone and hydrolysate. The structure of aglycone was determined by various colour reactions, alkaline degradation, neutral potassium permanganate oxidation, acetylation, methylation and I R superimposition by spectral data.

The hydrolysate, so obtained was neutralised and concentrated. The syrup was examined chromatographically. The sugar was identified to be glucose. The identity of sugar was confirmed by its  $R_f$  value and co-chromatography with the authentic sample.

The per-iodate oxidation of flavanone glycoside (Compound B) showed the consumption of 3.12 moles with the liberation of 1.16 moles of formic acid, which showed the presence of two sugar units, both in pyranose form. The position of glycosidic linkage in glycoside was determined as 7, 3' by direct comparison of its physical and chemical data with that of its aglycone. The glycoside was also hydrolysed

with the emulsin enzyme. From the various results of glycoside and its aglycone, the structure assigned to the compound (B) has been showed below.



4 - Hydroxy flavanone -7, 3' -O -  $\beta$  - D -  
Diglucopyranoside

The compound (C) ( a new glycoside), having molecular formula  $C_{40}H_{66}O_9$ , m.p.  $173^{\circ}C$  was isolated with ethyl alcohol extract of methanol from the leaves of *Butea monosperma* and was shown to be single entity by co-chromatography .

It gave colour reactions such as Liebermann Burchard Reaction , Ruzicka Reaction , Brieskorn Test and Zimmermann Test etc. for terpenoids and positive Molisch test.

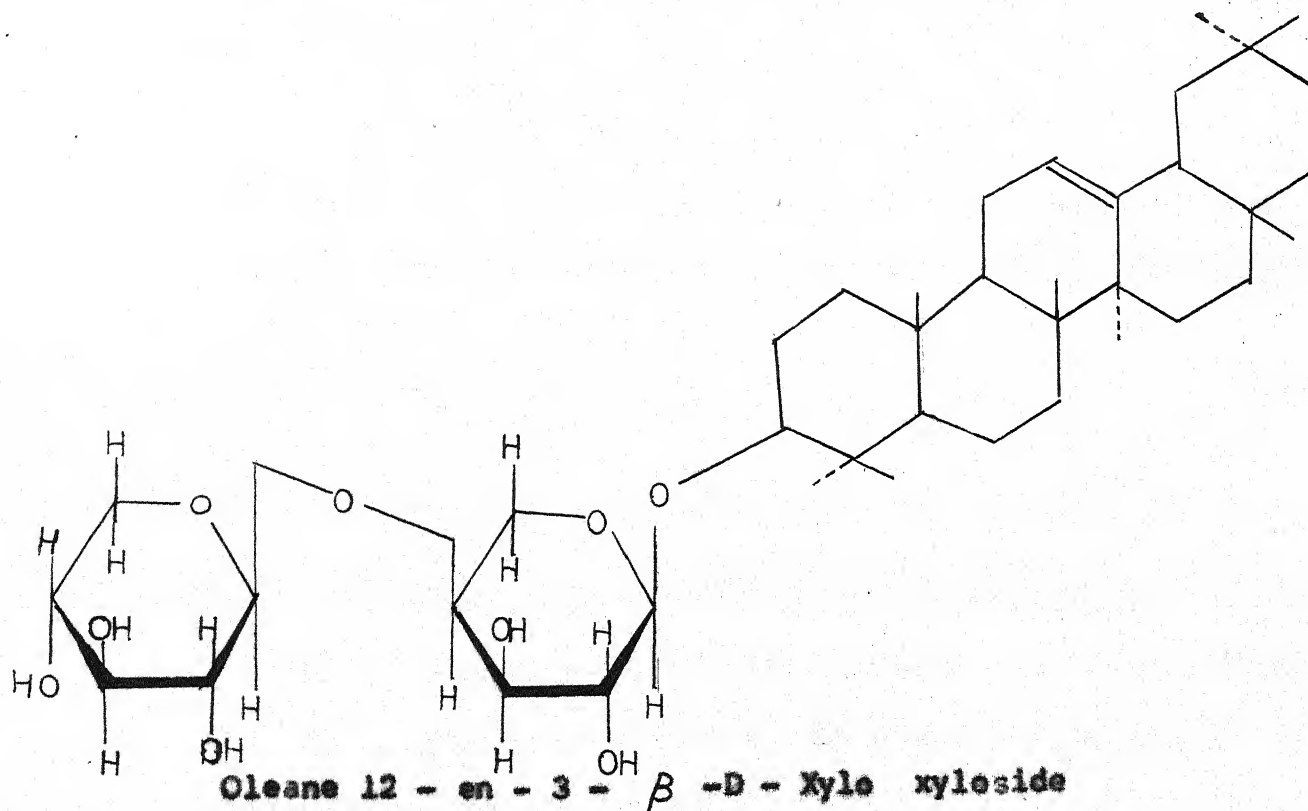
The compound (C) on acid hydrolysis with 7% methanolic sulphuric acid gave an aglycone which was confirmed by various colour reactions, alkaline hydrolysis, alkaline potassium permanganate oxidation, acetylation , methylation and Rf value in different solvents. The absorption maxima was found to be 205 nm.

The hydrolysate, obtained after acid hydrolysis was neutralised with  $BaCO_3$  and concentrated. The syrup was examined



chromatographically. The sugar was identified to be D-xylose. The identity of sugars was confirmed by their  $R_f$  values and co-chromatography with the authentic sample.

The per-iodate oxidation of the triterpenoid glycoside (Compound C) showed the consumption of 3.14 moles with the liberation of 1.2 moles of formic acid which was identified as 1 $\rightarrow$ 4 linkage between the two sugars in pyranose form. The position of glycosidic linkage in glycoside was determined at position -3 by direct comparison of its physical and chemical data with that of its aglycone. The position -3 was also supported by the Zimmermann test (for 3-keto group). The glycoside was also hydrolysed with the emulsin enzyme. From the various results of glycoside (C) and its aglycone, the structure assigned to the compound (C) has been showed below.



The compound (D), molecular formula  $C_{27}H_{30}O_{15}$ ,  $H_2O$ , m.p. 199-200°C was isolated with the ethylalcohol from the leaves of *Butea monosperma* and was shown to be single entity by co-chromatography.

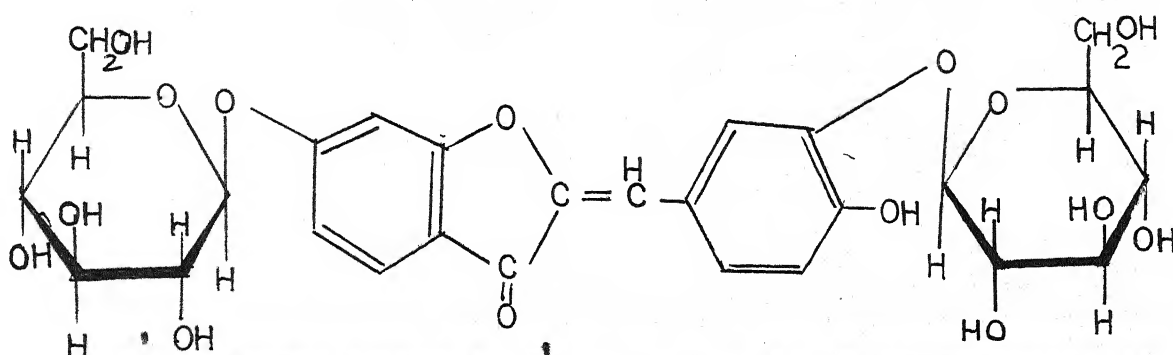
The compound (D) gave all colour reactions for aurone glycoside.

The compound (D), on acid hydrolysis with 7% aqueous ethanolic sulphuric acid, gave an aglycone which confirmed by various colour reactions, alkaline degradation, potassium permanganate oxidation, acetylation, methylation and  $R_f$  values in different solvents. Its identity was further confirmed by strong absorption maxima at 330 nm and 425 nm.

The hydrolysate, obtained after acid hydrolysis, was neutralised with  $BaCO_3$  and concentrated. The identity of sugar was determined by co-chromatography, m.m.p. and superimposition of I.R. as glucose.

The per-iodate oxidation of the aurone glycoside (Compound D) showed the consumption of 3.16 moles with the liberation of 1.2 moles of formic acid, which showed pyranose form. The position of glycosidic linkage in glycoside was determined at position 6, 3' by its physical and chemical data with that of its aglycone. The position -6 was supported by large hypsochromic shift and  $\lambda_{max}$  at 318 nm. and the position -3' was supported by I R peak at  $2850\text{ cm}^{-1}$ . The glycoside was also hydrolysed with the emulsine enzyme. From the various results of glycoside (D) and its aglycone, the structure assigned to the compound (D) has been showed below.

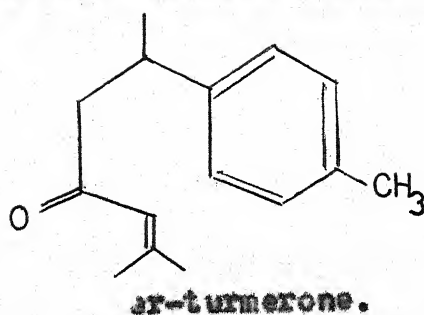




4- Hydroxy-Aurone-6, 3' - O-  $\beta$  - D - diglucoopyranoside.

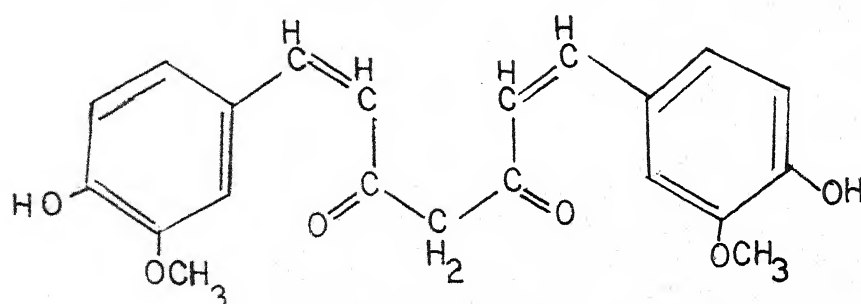
The third chapter deals with the survey of literature, isolation, purification and structural studies of two compounds (E) and (F). They were obtained from the rhizome of *Curcuma longa* with pet - ether and ethanolic extract respectively.

The compound (E), having molecular formula  $C_{15}H_{20}O$ , B.P.  $280-82^{\circ}C$ , gave all colour reactions of terpenoid and negative towards Molisch's test. The reduction with sodium borohydride and potassium permanganate oxidation of the compound (E) were in close agreement to the  $\alpha$ -turmerone. The identity of the compound (E) was finally confirmed by its absorption maxima which was found to be 237 nm and 263 nm in ethanol. The following structure has been assigned to be compound (E)



The compound (F), an orange yellow crystalline solid having molecular formula  $C_{21}H_{20}O_6$ , m.p.  $184^{\circ}-85^{\circ}C$ , was isolated with ethyl alcohol from the rhizome of *Curcuma longa*.

The compound (F) gave colour reactions and negative test towards Molisch's test. The acetylation, methylation, osmium tetroxide oxidation, alkaline degradation and Rf values in different solvents of compound (F) were in close agreement to the curcumin. The identity of the compound (F) was finally confirmed by its absorption maxima which was found to be 268 nm and 430 nm in ethanol. The following structure has been assigned to be compound (F).



Curcumin.

1,7 -bis (4 - hydroxy - 3 - methoxy phenyl) -1,6-heptadi-  
ene- 3,5 - dione

The fourth chapter deals with the survey of literature, isolation, purification and structural studies of three compounds (G), (H) and (I).

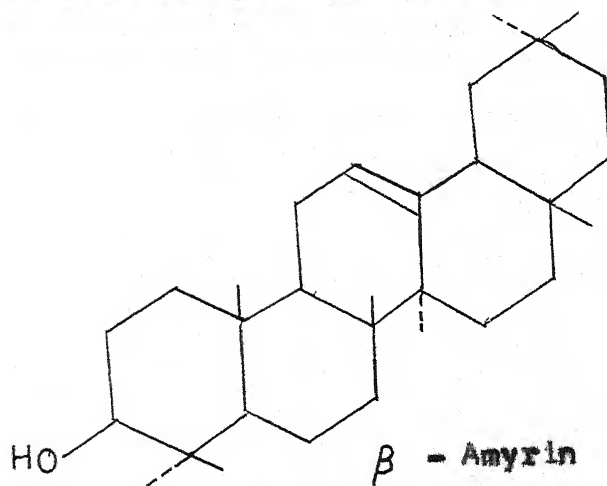
The compound (G), having molecular formula  $C_{30}H_{30}O$ , m.p.  $199^{\circ}C$ ,  $[\alpha]_D^{21} + 86^{\circ}$  was isolated with the ethyl acetate from the bark of *Ficus glomerata* and crystallised from hot ethyl acetate as a white shining needles. It gave colour reactions such as Liebermann Burchard Reaction, Neller test, Brieskorn test, Salkowski reaction and Zimmermann test etc. Its acetylated, benzoylated and oxidised products were prepared having m.p.  $236 - 37^{\circ}C$ ,  $[\alpha]_D^{23} + 81^{\circ}$ ; m.p.  $228^{\circ}C$ .



$[\alpha]_D^{25} -99.1^\circ$  and m.p.  $201^\circ\text{C}$  respectively .

On the basis of the above results obtained ,  
the compound (G) has been found to be  $\beta$  -amyrin.

The structure of the compound (G) ,  $\beta$  -  
amyrin has been assigned as follows.



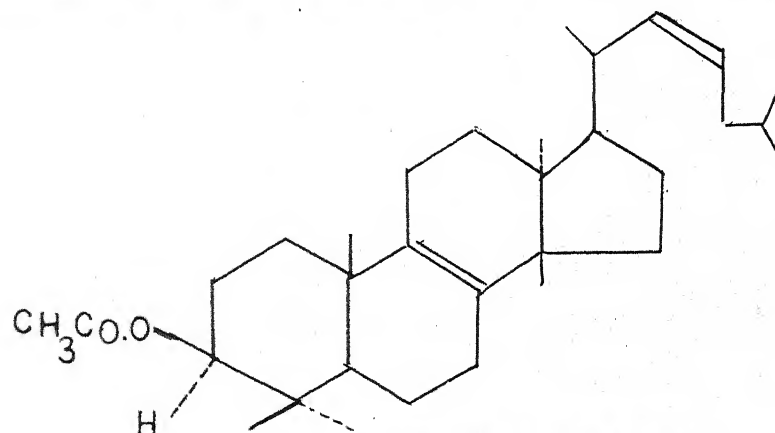
Oleane - 12 - en - 3 -  $\beta$  - ol

The compound (H)<sub>0</sub> having molecular formula  $\text{C}_{32}\text{H}_{52}\text{O}_2$  , m.p.  $180^\circ\text{C}$  ,  $[\alpha]_D^{23} + 0$  was isolated with ethanol from the bark of *Ficus glomerata* and recrystallised from methanol : chloroform (1:1 v/v) as a white crystalline needles.

The compound (H) gave colour reactions such as Liebermann Burchard reaction , Noller test, Salkowski reaction and Ruzicka test etc. Its hydrolysed and hydrogenated products were prepared having m.p.  $174^\circ\text{C}$  ,  $[\alpha]_D^{21} + 2^\circ$  and m.p.  $145^\circ\text{C}$  ,  $[\alpha]_D^{23} - 5^\circ$  respectively.

It was further confirmed by the acetylation and oxidation of the hydrolysed product (H<sub>1</sub>). The identity of the compound (H) was finally confirmed by its prominent IR peaks at  $1724$  and  $1244\text{ cm}^{-1}$ . The following structure has

been assigned to the compound (H) .

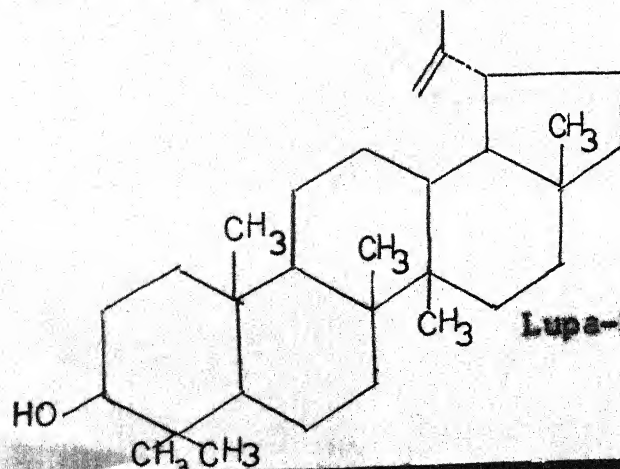


Gluanol acetate.

13  $\alpha$  , 14  $\beta$  , 17  $\beta$  -(H), 20  $\alpha$  (H)-lanosta-8, 22-diene-3- $\beta$  - acetate

The compound (I) ,having molecular formula  $C_{30}H_{50}O$  m.p.  $211-12^{\circ}C$ ,  $[\alpha]_D^{25} + 27.9^{\circ}$  was isolated with ethanol from the bark of *Ficus glomerata* .

It gave colour reactions such as Liebermann Burchard reactions, Salkowski reaction and Tschugajew reaction etc. and negative Molisch's test. The acetylation and chromic acid oxidation of the Compound (I) were in close agreement to the Lupeol. The identity of the compound (I) was finally confirmed by its I R absorption peaks at 1689, 885 and  $cm^{-1}$  and co-chromatography with its authentic sample . The following structure has been assigned to the compound (I).



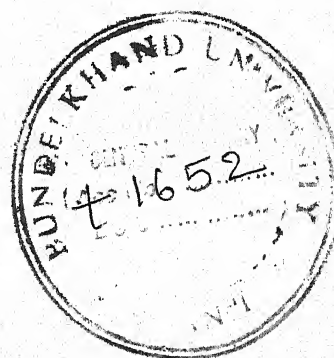
Lupeol

Lupa-20:29-ene-3-ol

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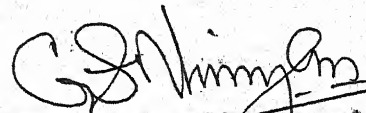


**C E R T I F I C A T E**  
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This is to certify that Km. Madhu Vashneya has completed all the requirements for her thesis entitled  
\* A STUDY OF CHEMICAL CONSTITUENTS OF CERTAIN MEDICINAL PLANTS OF BUNDELKHAND REGION \* for Ph.D. degree of Bundelkhand University, Jhansi under my supervision and guidance. She embodies the record of her own type. She has worked more than two hundred days in the Department of Chemistry (Chemical Lab.). Dayanand Vedic College, Orai, Bundelkhand University, Jhansi (U.P.).

Dated ORAI

March 25 ,1985.

  
(Dr. G.S. NIGAM)  
Deptt. of Chemistry,  
D.V. (P.G.) College ,

ORAI-295001.

## PREFACE

The present thesis entitled "A STUDY OF CHEMICAL CONSTITUENTS OF CERTAIN MEDICINAL PLANTS OF BUNDELKHAND REGION" has been consolidating the research work into four chapters has its individuality in dealing with different aspect of Chemistry of Natural products.

The first chapter has the information of the historic and general views of Indian medicinal plants covering the literature survey upto 1983.

The second chapter deals with the chemical examination of leaves of *Butea monosperma*. Alactone, flavanone glycoside, triterpenoid glycoside and aurone glycoside from this plant.

The third chapter describes the chemical examination of the rhizome of the plant *Curcuma longa* for one sesquiterpene (essential oil) and one colouring matter. Their structures were confirmed by synthesis and spectral data.

The fourth chapter reveals the chemical examination of the bark of *Ficus glomerata*. The triterpenes were isolated and their study was done by degradative and spectral studies.

The work presented in the thesis has been done in the chemical laboratory Dayanand Vedic Postgraduate College, ORAI under the supervision of Dr. G.S. Niranjan D.Phil, FICS Department of Chemistry, Dayanand Vedic Postgraduate College, ORAI.

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I am obliged to the authorities of the C.D.R.I. Lucknow and the I.I.T. Kanpur for their valuable help in recording the I.R. , U.V. and N.M.R. Spectra of the compounds.

It is my privilege to record my gratitude to my all family members particularly to my brother Mr. And Mrs. Ashok Varshneya and sister and brother-in-law Mr. & Mrs. Dr.R.N. Gupta, Mr. & Mrs. Rajesh Gupta for their inspiration which enabled me to go through this difficult path. I also tender my heartiest thanks to Mrs. Dr. G.S. Niranjan and Mrs. Dr. S.P. Saksena for their affection rendered during the research period.

The author expresses her gratitude to her parents Sri & Smt. Vijai Chandra Varshneya for their encouragement.

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MARCH 25 ,1985.

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CHAPTER - I

INTRODUCTION



One of the essential requirement for the rapid economic growth of any nation is to develop its own natural resources. Fortunately, India is gifted with varying climatic conditions and variety of plants and vegetations which form an important group of valuable resources. Hence crude plant products have been in use for medicinal purposes in this region since time immemorial. It is, therefore, natural for scientists engaged in this area to work hard for the study of various aspects of the medicinal plants. The isolation and structural study of chemically pure active compounds lead us to their synthesis and new sources which advances the cause of medicine.

During the last few decades a systematic chemical investigation in the field of Natural Products, received a world wide attention with the development of modern physico-chemical technique like chromatography, U.V., I.R., N.M.R., Mass. Inspite of the fact that on the line of above modern technique, an extensive work has been done on the chemistry of Plant Products and the pharmaceutical properties of a large number of plant constituents has been studied in greater detail, there are still a family of large number of Indigenous medicinal plants which have not been investigated thoroughly. The Ayurvedic system of medicine<sup>1</sup> in India describes the medicinal use of several hundred plant species in the treatment of various human ailments. The Unani system of medicine also incorporates the use of rich medicinal flora of our country.

The plant products obtained from the plants are classified into the following groups :-

(1) Alkaloids (2) Terpenes (3) Glycosides (4) Saponins (5) Antho-

cyanins (6) Flavanones and Flavones (7) Essential oils (8) Fatty oil and waxes (9) Lactones and Higher alcohols (10) Steroids (11) Carotenoids (12) Polyphenolic compounds (13) Polysaccharides (14) Higher hydrocarbons (15) Sugars (16) Lipids (17) Aminoacids and Proteins etc.

The classification is not rigid in the sense that one compound may be said to belong to more than one group according to their molecular structure. A brief account of the review on the classes of compounds investigated from the plants which have been incorporated in the present thesis is described below :-

- (i) Terpenoids
- (ii) Flavanoids
- (iii) Lactones
- (iv) Essential oils

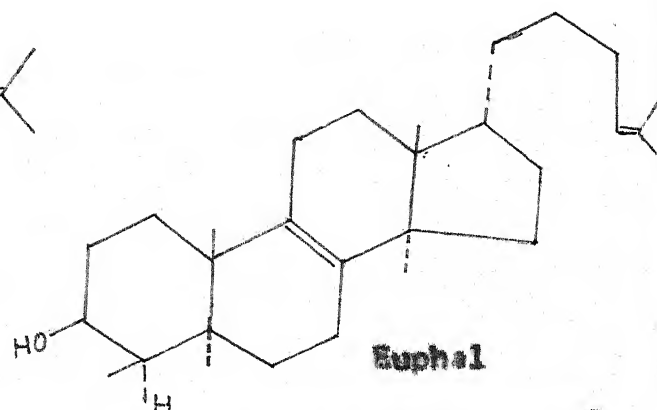
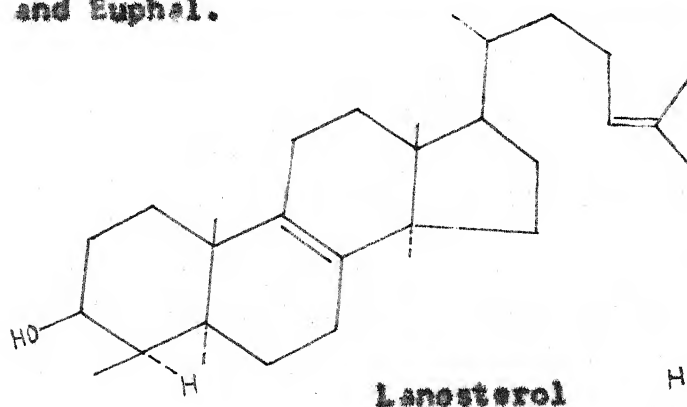
(i) TERPENOIDS :-

Terpenoids are liposoluble, polycyclic and hydro-aromatic alcohols. They are widely distributed in the Natural products and occurred in plants in the form of esters and glycosides or in the free state. The monocyclic or dicyclic triterpenes are not reported now-a-days. Tricyclic triterpenes are also rare and one important compound in this group is ' ambicin '. Most of the known triterpenoids have been found to either tetracyclic or pentacyclic structures.

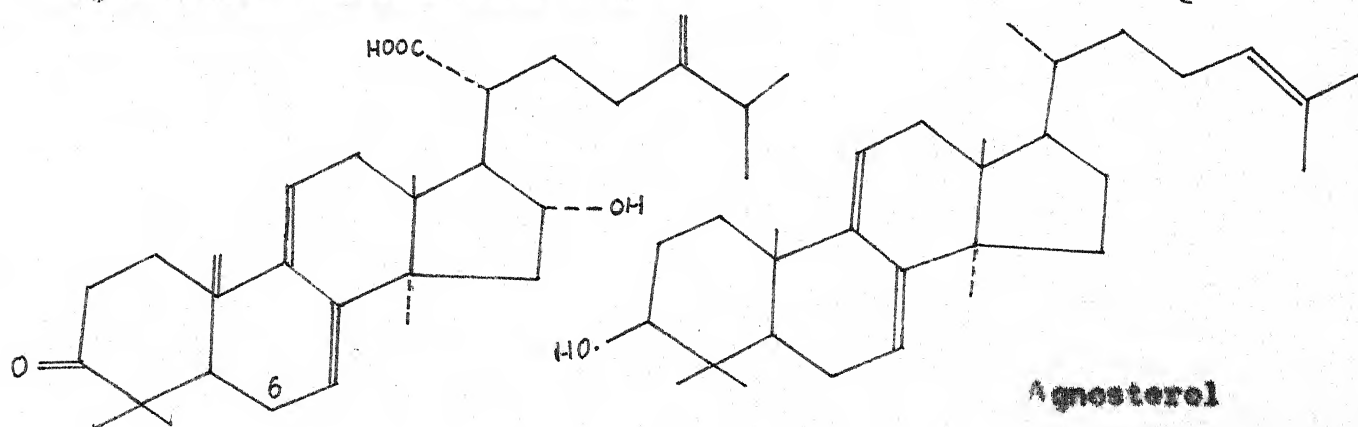
TETRACYCLIC TRITERPENOIDS :-

They resemble to steroids particularly in biogenetic relationship and they are of great importance and

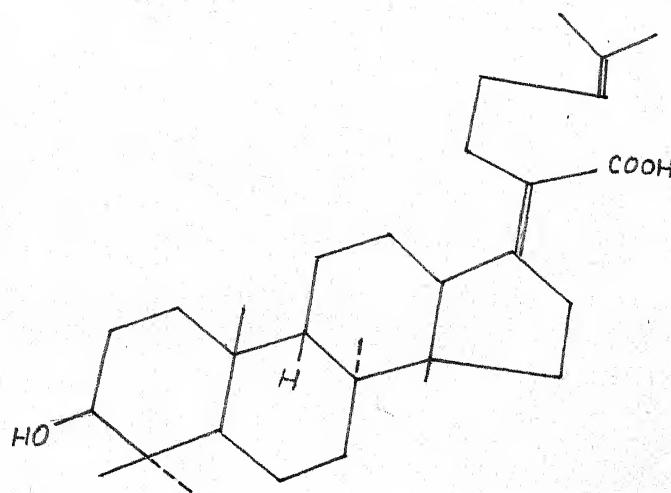
also of interest. There are two main families of this group of compounds which can be represented by two substances Lanosterol<sup>2</sup> and Euphal.



The common members of these classes are polyporenic acid<sup>3</sup>, agnosterol<sup>4</sup> and eburicolic acid<sup>5</sup>.



*polyporenic acid*



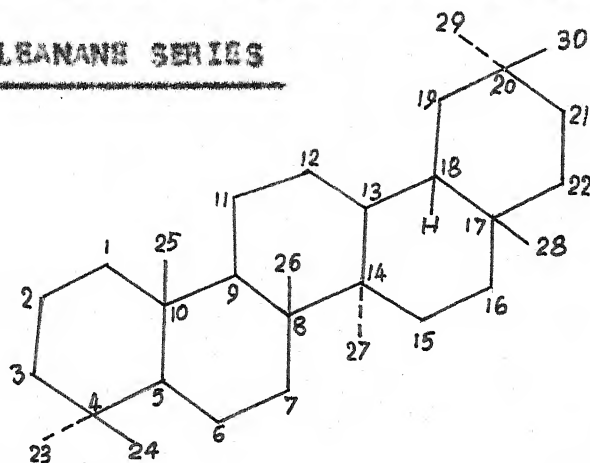
Eburicolic acid -4- contd....



## PENTACYCLIC TRITERPENE

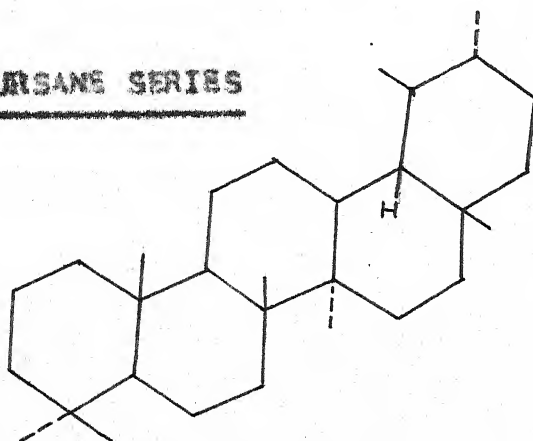
They are obtained in plants in free state and in combined state as glycosides (Saponins). The non-glycosidic triterpenoids are found as excretion and protected by cuticle. On the basis of chemical structures of their classes they can be further divided into three main groups.

### (I) OLEANANE SERIES



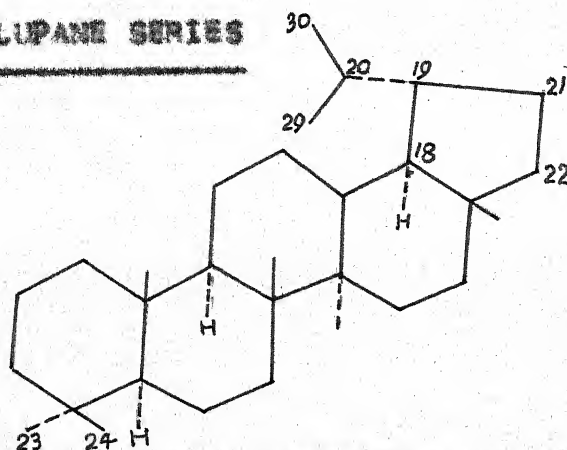
OLEANANE

### (II) URSANE SERIES



URASANE

### (III) LUPANE SERIES



LUPANE

The pentacyclic triterpenoids are generally oxygenated at position-3 and in most of the cases  $\beta$ -hydroxy group is a functional group which is acetylated easily. In few cases, an  $\alpha$ -hydroxyl group as in boswellic acid or a ketonic group as in icterogenin may occur. The nucleus may be saturated or may contain one or even more ethylenic linkage. A carboxylic group frequently occur at C<sub>17</sub>.

Various triterpenes have also been found to be physiologically active.

In the present thesis the terpene glycosides and terpene aglycones have been isolated from *Eutea monosperma* and *Ficus glomerata* respectively.

#### FLAVONOIDS -

Flavonoids are naturally occurring pigments. These contain two benzene rings which are linked by a propene bridge ( $C_6 - C = C - C_6$ ) except in isoflavanones in which the arrangement is  $C_6 - C - C - C$ . It includes chalcones, dihydrochalcones, flavones, leucoanthocyanidins and anthocyanidines, flavanols, auronas and isoflavones.

The flavonoids are yellow pigments and are found in various parts of the plants. They occur in free state or in the form of glycosides. Generally the diglycosides of the flavones are found in the plants which are called biosides.

The plants have many physiological active compounds which refer to flavonoids and other relative compounds. The

flavanol glycoside 'Rutin' has been described for its therapeutic properties. The insectisidal action of polyhydroxy flavanones and their ethers and the action of flavanones on isolation with an enzyme system have been studied<sup>6</sup>.

#### HYDROXYLATION PATTERN IN FLAVANONES

Generally in the flavanones the hydroxyl groups occur at 5,7,3 and 4' positions. The progressive increase in hydroxylation causes relatively large shift in colour maxima.

##### FLAVANONES

λ Max nm.

Dihydroxy quercetin

300 nm.

#### METHYLATION PATTERN IN THE FLAVANONES

Methylation in flavanones lower the value of absorption maxima. The most common methylated portion in flavanones are 4' , 7, and 8.

##### FLAVANONES

λ Max nm.

7-hydroxy - 4' - methoxy

277 nm.

5-hydroxy - 7 - methoxy

290 nm.

7-hydroxy - 5,8-dimethoxy

287, 325 nm.

5,7-dihydroxy- 8 -methoxy

292, 340 nm.

#### GLYCOSYLATION PATTERN IN FLAVANONES

The majority of flavanones have been found in free state. Only a few flavanones have been reported as glycosides. The reported glycosides are 4' , 7-dihydroxy - 4'-glycoside; carthamin as a glucoside taxafolin, isocarthamin as a glucoside taxifolin liquiritigenin- 4'glucoside and 7-rhamno glucoside etc.

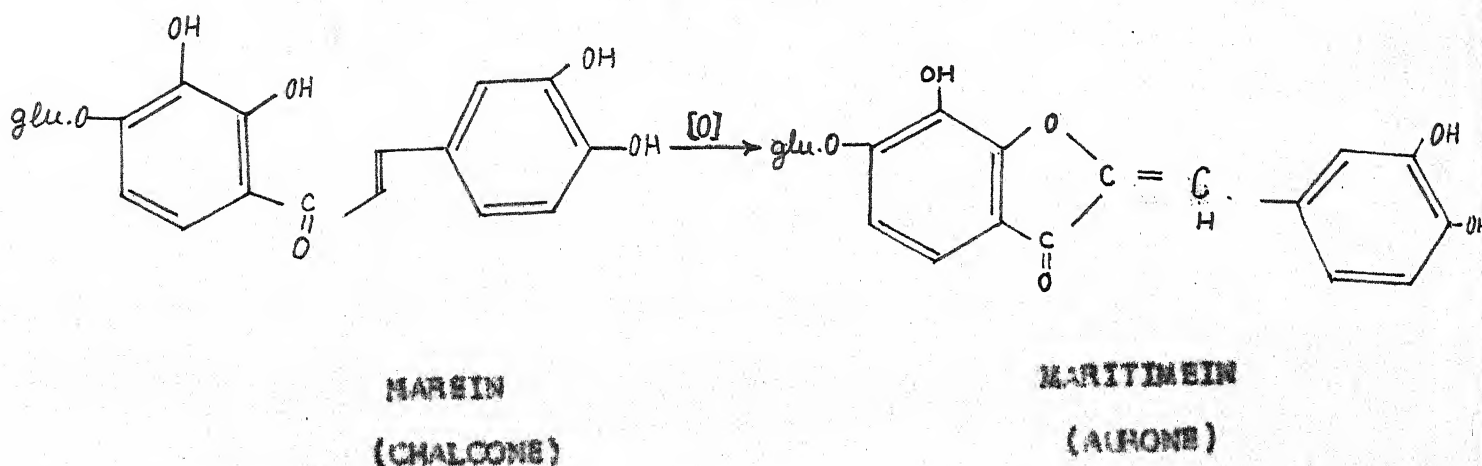


The author has been even to isolate a flavanone diglycoside from the leaves of *Butea monosperma*. The chemical examination of above compounds has been described in the chapter second of the thesis.

#### AURONES :-

In 1943 , Geissman<sup>7</sup> discovered aurones which have the fundamental nucleus benzylidene coumaran-3-one. These compounds are usually orange coloured and are isomeric with flavones. The nuclear hydroxylation pattern of Aurones resembles to chalcone as well as flavones. The relationship between flavones and aurones are indeed very close, not only in structural features and distribution in plants, but also chemically.

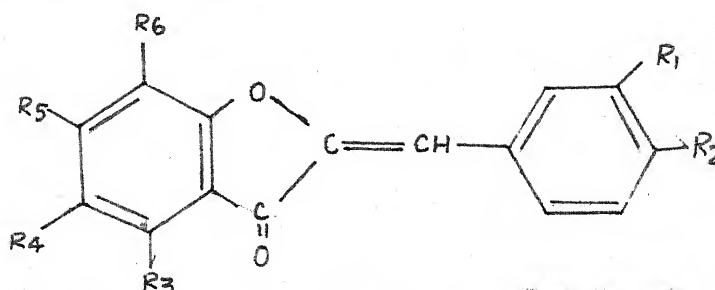
Aurones and flavones are also related through chalcones. When cyclised in acid media these afford flavones, treated with base they may yield aurones. Moreover some chalcones are oxidised by air to give aurone directly.<sup>8,9</sup>



#### HYDROXYLATION PATTERN IN AURONES

All known aurones have hydroxyl or methoxyl group at 3' , 4' and 6-positions. The progressive increase in

hydroxylation causes relatively large shift in the colour maxima which are listed below -



	1st band	2nd band
(i) $R_2 = OH, R_1 = R_3 = R_4 = R_5 = R_6 = H$	257 nm	397 nm.
(ii) $R_1 = R_2 = OH, R_3 = R_4 = R_5 = R_6 = H$	259, 277 nm	413 nm.
(iii) $R_4 = R_6 = OH, R_1 = R_2 = R_3 = R_5 = H$	283 -	-
(iv) $R_5 = R_6 = OH, R_1 = R_2 = R_3 = R_4 = H$	317, 379	444 nm.
(v) $R_1 = R_2 = R_5 = R_6 = OH, R_3 = R_4 = H$	412	-
(vi) $R_1 = R_2 = R_5 = OH, R_6 = OCH_3, R_3 = R_4 = H$	406 nm.	-

#### METHYLATION PATTERN IN AURONES

This pattern also occupies the same positions as hydroxyl groups but exception of 7-hydroxyl group. Methylation of phenolic group does essentially affect the spectra of aurones.

#### GLYCOSYLATION PATTERN IN AURONES

Aurones occur in the plant with sugar residue attached in 6, 3' and 4' -positions. In aurones chelation and hydrogen bonding are such factors which affect the spectral shift. The spectra of natural aurone glycosides are very closely related

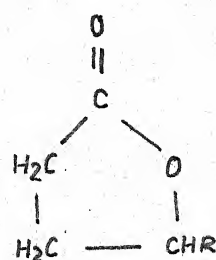
to the spectra of their aglycones with the margin of 3-6 nm shift towards the visible region.

In the present thesis one aurone diglycoside and one flavanone diglycoside have been isolated from the leaves of *Butea monosperma* and their structures were identified with the help of spectral studies.

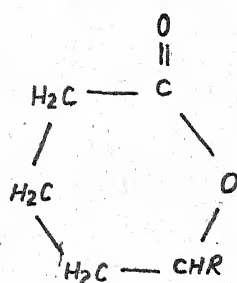
### LACTONES

The different kinds of hydroxy acids undergo dehydration in different base to form an internal esters, called lactone. The product, which obtained from the particular hydroxy acids, depends upon the location of -OH group ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$ ) with respect to -COOH group.

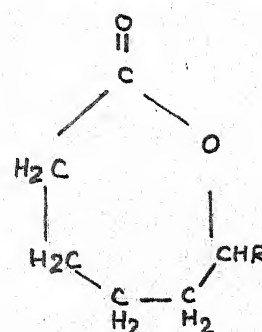
When a  $\alpha$ -hydroxy acid is heated, it loses water by the process of esterification, which takes place in such a way as to form a six membered ring, the product is called lactide. A  $\gamma$ - or  $\delta$ -hydroxy acids also loses water by esterification but the time reaction occurs with in a single molecule to yield a cyclic ester known as a lactone. Lactonisation occurs spontaneously to give an equilibrium mixture, i.e. - chiefly lactone treatment with base rapidly opens the lactone ring to give the open chain salt.



$\gamma$  - Lactone  
(Acyclic ester)  
(Five membered ring)



$\delta$  - Lactone  
Acyclic ester  
(Six membered ring)



$\epsilon$  - Lactone  
Acyclic ester  
(Seven membered ring)



$\gamma$  - and  $\delta$  - lactones are also formed when  $\gamma$  ,  $\delta$  or  $\delta$  or  $\delta\epsilon$  unsaturated acids are treated with concentrated sulphuric acid.

The natural lactones are obtained generally from plants during defattation . Sometimes lactones may occur by extraction from benzene, acetone, ethanol etc.

#### ESSENTIAL OIL-

The essential oils<sup>10</sup> are steam volatile substances and may be defined as odouriferous substances of oily nature obtained from vegetable sources. These are generally liquids and sometimes semisolids or solids at room temperature and volatilises without decomposition. Some of the essential oils were found to be decomposed during distillation.

Essential oils occur in whole parts of the plants but in several cases it is restricted only to some special portion of the plants such as leaves, barks, roots, flowers and fruits. Such as essential oils in the plant, *Ocimum sanctum* , belongs to the family Labiatae, is found in most of its parts, the whole of the plant , where as the rose oil is present only in flower , cinnamon oil is confined to the bark, leaves and little in the root. The essential oils are composed of a number of chemical compounds such as hydrocarbons , alcohols , esters , aldehydes , lactones , oxides , ketones and occasionally compounds containing nitrogen and sulphur.

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## CHAPTER - II

### CHEMICAL EXAMINATION OF BUTEA MONOSPERMA



The plant *Butea monosperma* is commonly known as Dhak or Palas, belongs to family Leguminosae. It is a moderate or small sized perennial tree. Trunk crooked, leaves are long, deciduous, rigidly coriaceous, glabrescent above, silky tomentose and strongly veined beneath.

The plant *Butea monosperma* has very important medicinal values as described below.

The leaves are astringent, diuretic tonic and aphrodisiac. They are given in diarrhoea, heart burn, sweating of phthisis, diabetes, flatulent colic, piles, glycosuria. Their infusion or decoction is given as a rectal enema in diarrhoea and dysentery, as a vaginal douch in leucorrhoea and as a mouth wash for septic and congested throat. A hot poultice of the leaves is applied to disperse boils, pimples, tumorous piles, ulcers, buboes and swelling etc. In retention of urine the pubic region is fomented with the leaves.

A paste of the seeds is given in ring worm. The pulverised seeds are used for killing maggots in wounds and sores, the powder is also a rubifacient.

Flowers of the plant have the same medicinal importance as leaves. From the literature, the plant was surveyed and the details of work done are given below.

CONSTITUENTS	PART OF THE PLANT	REFERENCES
(1) $\alpha$ -Amyrin, $\beta$ -sitosterol, $\beta$ -sitosterol- $\beta$ -D-glucoside and sucrose	Seeds	J.I. Pharm., 39(4), 79-80 (1977) (Eng)

(ii) $\gamma$ -Heneicosanoic acid $\delta$ -lactone	Seeds	Planta Med. <u>35</u> (2), 286-8 (1979) (Eng).
(iii) Monospermin (Alkaloid)	Seeds	Chem. Ind. , <u>3</u> , 98(1981)
(iv) Butein and Butin	Flowers	Proc. Indian Acad. Sci., <u>12</u> (A), 477-80 (1940).
(v) Butrin , Butin and Butein	Flowers	Proc. Indian Acad. Sci., <u>13</u> (A), 395-98 (1941).
(vi) Palasitrin, Isobutrin, Butrin	Flowers	J. Chem. Soc., 1589-92 (1935).
(vii) Inide	Seeds	Chem. Ind. (London) <u>43</u> , 1376 (1970).
(viii) Aleuritic acid	Resin	Indian J. Chem., <u>5</u> (5), 182-4 (1967) (Eng ).
(ix) Palasonin	Seeds	Indian J. Chem. <u>5</u> (2), 86-7 (1967) (Eng ).
(x) (7 Flavonoid glucoside) Butrin , isobutrin, ceroepsin, isoceroepsin, sulfurein, monospermoside and isomonospermoside.	Flower	Phytochemistry <u>9</u> (10), 2231-5 (1970) (Eng).
(xi) Proanthocyanidin	Bark, Gum	Indian J. Chem. <u>9</u> (11), 1201-3 (1971) (Eng).

Since , out of all the parts of the plant ,the leaves have more medicinal importance and no work has been

reported in the literature on the leaves of the plant.

Therefore, it is worthwhile to study its chemical constituents for their molecular structures and pharmaceutical values.



EXTRACTION AND ISOLATION OF CHEMICAL CONSTITUENTS  
OF LEAVES OF BUTEA  
MONOSPERMA.

The leaves of the plant *Butea monosperma* were locally collected and identified for their authenticity in Botany Department, D.V. (P.G.) College, ORAI (U.P.).

2 kg. dried leaves of *Butea monosperma* were extracted with petroleum ether (60-80°) in a soxhlet extractor for 36 hours in different lots for defatting. The defatted leaves were exhaustively extracted with 95 % hot ethanol in 5 litre flask in different lots until a very faint coloured extract was obtained. The total ethanolic extract was concentrated to a very small volume.

The concentrated extract was kept in a refrigerator for seven days. A white solid mass was settled down. It was filtered washed with ethanol and dried in a vacuum desiccator. It was crystallised from chloroform which gave pure and crystalline white compound (A).

The syrupy mass extract was refluxed with solvent ether in a solvent - solvent extractor in different lots approximately 200 ml. each which was concentrated upto 50 ml. under reduced pressure where upon a grey coloured mass was obtained. It was chromatographed by column chromatography using silica gel-G as adsorbent and petroleum ether : ether (1:4 v/v) as eluent. An orange - yellow compound (B) was obtained. It was recrystallised from hot water thrice and followed by ethanol.

The remaining grey coloured mass was refluxed with methanol in different lots. The whole extract was concentrated in small volume under reduced pressure. The whole extract was kept in a friz for seven days. A white solid settled mass was

separated by filtration. It gave single spot on paper chromatography and thin layer chromatography using silica gel-G and *n*-butanol : acetic acid : water ( 4:1:5 v/v) as solvent. It was then crystallised from hot ethyl acetate, named as compound (C).

The above filtrate was further concentrated and column chromatographed using magnasol as an adsorbent and methyl alcohol as eluent, which on concentration gave a brownish red coloured mass. The mass on crystallisation from butyl alcohol afforded a crystalline compound (D) which indicated single spot in thin layer chromatography.



Defatted with Petroleum ether  
( 60-80° ) in a soxhlet for 36 hours

Defatted material

Extract

Refluxed with 95% ethanol

Extracted material

Extract

Concentrated and kept  
in a Friz and filtered

A white compound  
( A )

Filtrate

Extracted with solvent  
ether and filtered

Extract

Residue

Extracted with methanol and  
kept in a Friz for 7 days and filtered

A white solid compound  
( C )

Extract

Concentrated and  
chromatographed over  
magnasol column and  
eluted with methanol

A brownish red colour solid  
Compound  
( D )

An Orange yellow solid

Compound  
( n )

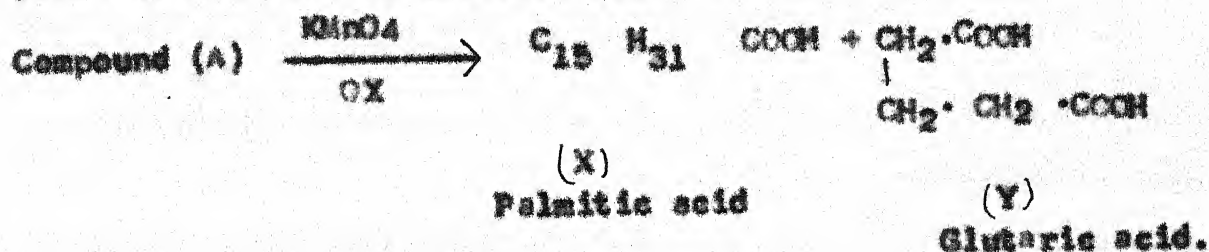
Chromatographed over  
silica gel-G column and  
eluted with Pet-ether  
(1:4 v/v)

CHEMICAL STUDY OF COMPOUND - (A)

A white colourless crystalline compound (A), having molecular formula  $C_{21}H_{40}O_2$ , m.p.  $70-71^\circ C$ , isolated with the ethanolic extract from the leaves of *Butea monosperma*. The compound (A) was soluble in benzene, ether and its homogeneity was checked by thin layer chromatography and paper chromatography and methanol : chloroform as developing solvent and 10% sulphuric acid as spraying reagent.

The compound (A) on saponification<sup>1</sup> followed by acidification was reprecipitated unchanged showed the presence of a lactone ring in it. The I R peak at  $1735\text{ cm}^{-1}$  and NMR signal at  $\tau\ 4.9^{2,3}$  evidently showed that the lactone was found to be  $\delta$ -lactone.

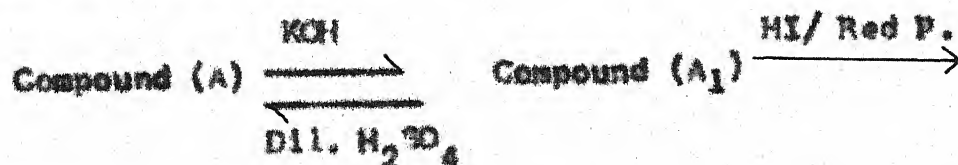
The compound (A) did not decolourised potassium permanganate solution or bromine water, indicating it, to be a saturated compound. It showed the absorption maxima at 212 nm in the UV region and also gave a red colour with 2:4 - di nitrophenyl hydrazine showing the presence of  $>C=O$  group in the compound (A) which was further confirmed by I R spectral data at  $1680\text{ cm}^{-1}$  ( $\gamma\ C=O$ ). It was found to be aliphatic in nature as revealed by the I R absorption band at  $1735\text{ cm}^{-1}$ . On oxidation with potassium permanganate it gave two acids i.e. palmitic acid (X) and glutaric acid (Y).



The formation of glutaric acid can only be explained on the basis of the presence of a  $\delta$ -lactone ring in the compound (A)

The I R spectrum should a doublet at  $735\text{ cm}^{-1}$  and  $725\text{ cm}^{-1}$  and which a characteristic of polymethylene group in long chain. The NMR spectrum showed a diagnostic signal at  $\tau$  8.70 (multiplet) for methylene proton. The spectral data revealed that compound (A) was a straight<sup>4</sup> chain methylene compound. The above facts suggested that the compound (A) has a straight polymethylene chain.<sup>2,5</sup>

The compound (A) on alkaline hydrolysis with potassium hydroxide gave a monohydroxy higher fatty acids ( $A_1$ ), m.p.  $80-82^\circ\text{C}$ . It was treated with dilute sulphuric acid which gave the original compound (A). On reduction with hydroiodic acid and red phosphorus, the acid ( $A_1$ ) produced a higher fatty acid identified to be  $n$ -heneicosanoic acid having molecular formula  $\text{C}_{21}\text{H}_{42}\text{O}_2$ , m.p.  $74^\circ\text{C}$ . Its identity was confirmed by m.n.p., co-chromatography and super imposition of I R spectra with its authentic sample.



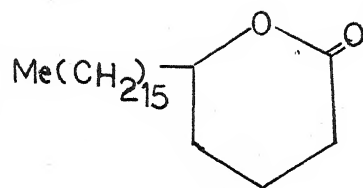
$n$ -Heneicosanoic acid.

The NMR spectrum of the compound (A) also confirmed it to be a long chain  $\delta$ -lactone. It showed triplet (3 H) at  $\tau$  9.1 for methyl proton (due to terminal methyl group)<sup>6,7</sup>. The NMR signal at  $\tau$  8.7 (3  $\text{CH}_2$ ) and  $\tau$  8.4 (4 H) for methylene protons ( $\beta$  to  $-\text{CHO}$ ),  $\tau$  6.75 (2 H) for methylene



protons (  $\alpha$  to  $>C=O$  ) and  $\tau$  4.9 ( 1 H ).

Thus on the basis of the above evidences the compound (A) has been assigned to be  $\delta$  - lactone of  $n$  - heptacosanoic acid. Thus the structure of the compound (A) is as follows.



8 - lactone of  $\alpha$ -tetracosanoic acid

## EXPERIMENTAL

### 1. EXTRACTION AND ISOLATION

The compound (A) was isolated with ethanolic extract from the leaves of *Butea monosperma* as described on page 15. The compound (A) was purified with chloroform and recrystallised from ethanol : chloroform (1:1 v/v) as a white flakes, having molecular formula  $C_{21}H_{40}O_2$ , m.p.  $70-71^{\circ}C$ .

### 2. ELEMENTAL ANALYSIS

The compound (A) on elemental analysis gave the following results.

Anal. data found (%)	Calcd. for $C_{21}H_{40}O_2$
C = 77.5	C = 77.77%
H = 12.4	H = 12.34%
$M^+ = 326$	$M^+ = 326$

### 3. THIN LAYER CHROMATOGRAPHY

Thin layer chromatography was carried out on the plates coated with silica gel-G using methanol : chloroform (1, 11) mixture in a different ratio as an irrigating solvent.

(i) Methanol : Chloroform (1 : 1 v/v) mixture.

(ii) Methanol : Chloroform (2:3 v/v) mixture

The chromatoplate was dried and sprayed with 10% sulphuric acid. Single spot was obtained which showed the purity of the compound (A).

#### 4. CHARACTERISTIC REACTIONS

The compound (A) gave following characteristic reactions.

- (i) As it did not give nitrate test so it was aliphatic in nature.
- (ii) As it formed red colour with 2:4-dinitrophenyl hydrazine indicating the presence of ketonic group in compound (A).
- (iii) It did not decolourise potassium per manganate solution or bromine water indicating the saturated nature of compound (A).

#### (iv) SAPONIFICATION<sup>1</sup> FOLLOWED BY ACIDIFICATION -

A mixture of compound (A) ( 1 gm.) and 50 ml. of N/2 alcoholic KOH in 250 ml. round bottom flask attached with reflux condenser, was heated gently on a water bath for 2-3 hours until hydrolysis was completed. The cooled solution was poured in 50 ml. distilled water through condenser. The solution was titrated excess of alkali with standard 0.5N hydrochloric acid ( 25 ml.) using phenolphthalein as indicator. The completion of reaction was indicated by faint pink colour at end point.

#### 5. POTASSIUM PERMANGANATE OXIDATION OF COMPOUND (A) -

Compound (A) (50 mg) with 5 % aqueous potassium per manganate solution (5 ml) and 10 % sodium hydroxide solution (5 ml) was refluxed on water bath for 10 hours. After oxidation , the reaction mixture was cooled and acidified with dilute hydrochloric acid . The excess of



manganese dioxide was removed by the addition of sodium bisulphite. The solution was filtered, concentrated and kept in a refrigerator. When a colourless solid (X) separated out, it was filtered off. The solid was washed well with distilled water and dried at room temperature. It was recrystallised from ethanol to a crystalline compound (X), having m.p.  $61-61.5^{\circ}\text{C}$ . It was found to be Palmitic acid<sup>8</sup> (Lit. m.p.  $62^{\circ}\text{C}$ ). It was confirmed by m.m.p., co-chromatography and its amide formation, m.p.  $105-106^{\circ}\text{C}$  (Lit.<sup>9</sup>  $106-107^{\circ}\text{C}$ ).

The aqueous filtrate was extracted with solvent ether. The ether extract on evaporation gave another solid compound (Y). The compound (Y) was recrystallised from benzene to give a white needles having m.p.  $96-97^{\circ}\text{C}$  which correspond to m.p. of glutaric acid<sup>8</sup> (Lit. m.p.  $97^{\circ}\text{C}$ ). Its identity was confirmed by m.m.p. and co-chromatography with its authentic sample. It was further evidenced by its diamide, m.p.  $174-175^{\circ}\text{C}$  (Lit.<sup>9</sup>  $176-177^{\circ}\text{C}$ ) and anhydride derivative, m.p.  $55^{\circ}\text{C}$  (Lit.<sup>9</sup>  $56^{\circ}\text{C}$ ).

## 6. ALKALINE HYDROLYSIS OF COMPOUND (A) -

6.1 The compound (A) (50 mg.) was refluxed with 7% ethanolic potassium hydroxide (50 ml.) solution on water bath for 10 hours. After cooling the solution, the excess of ethanol was distilled off under reduced pressure and residual content was poured in the distilled water to yield a white precipitate. The precipitate was filtered off, washed well with water, dried and recrystallised from benzene to give compound ( $A_1$ ), m.p.  $80-82^{\circ}\text{C}$ .

## 6.2 REDUCTION OF HYDROLYSED PRODUCT $-(A_1)$

The hydrolysed product ( $A_1$ ) (20 mg) was dissolved in the freshly distilled hydroiodic acid (5 ml) and a pinch of red phosphorus was added as a catalyst. The mixture was refluxed on a water bath for 5 hours. It was cooled and poured in a distilled water. It was filtered and extracted with solvent ether. The ether on drying gave a product which on crystallisation from methanol gave a crystalline product having molecular formula  $C_{21}H_{42}O_2$ , m.p.  $74^\circ C$  (Lit<sup>10</sup>  $74.3^\circ C$ ). It was identified as  $n$ -heneicosanoic acid by m.m.p., co-chromatography and super imposition of I R spectrum with its authentic sample.

## 7. ABSORPTION SPECTRUM -

### (i) U.V. SPECTRAL DATA .

$\lambda_{max}$  . 212 nm.

### (ii) I R SPECTRAL DATA (KBr) $cm^{-1}$

2910, 1735, 1465, 1430, 1310, 1250, 1230, 1215, 935,  
735 and  $725\ cm^{-1}$

### (iii) NMR SPECTRAL DATA ( $\tau$ ppm) ( $CCl_3$ )

The NMR spectrum was recorded on Varian A-60 spectrometer. The shifts are denoted in  $\tau$  value

Signal in ( $\tau$ ppm)	Assignment
4.9	(1 H) $\delta$ -lactone band
6- 7.5	(2 H) Methylene proton ( $\alpha$ to $>C=O$ )
8.4	(4 H) Methylene proton ( $\beta$ to $-CHO$ )
8.7	Methylene proton
9.1	Methyl proton .

CHEMICAL STUDY OF COMPOUND - (B)

The compound (B), an orange yellow crystalline solid, molecular formula  $C_{27}H_{32}O_{15} \cdot 2H_2O$ , m.p.  $190^{\circ}C$ , was isolated with the ethereal extract from the leaves of *Eutea monosperma* as described on page 15. The compound (B) was crystallised from hot water and ethanol, a tiny colourless glistening needles was obtained. Its purity was checked by thin layer chromatography and paper chromatography. Single spot was obtained on Whatmann No. 1 filter paper and thin layer chromatoplate. The compound (B) was dehydrated in oven at  $120^{\circ}C$  for 15 hours. It was slightly soluble in cold water and moderately soluble in hot ethyl alcohol, methyl alcohol, glacial acetic acid and very readily soluble in pyridine.

The molecular weight of the compound (B) was further confirmed by elemental analysis and molecular weight determination.

The compound (B) gave Molisch's test but it neither reduced Fehling's solution nor Tollen's reagent, indicated the presence of compound (B) as a glycoside and the sugar is not in free state.

The compound (B) was hydrolysed with 5% dilute sulphuric acid, gave an aglycone and an aqueous hydrolysate. The hydrolysate gave a brown colour spot with aniline hydrogen phthalate<sup>11</sup> and reduced Fehling's solution and Tollen's reagent showed the presence of free sugar in the hydrolysate and the compound (B) as a glycoside.

The separate chemical examination of the aglycone and sugar moiety, confirmed the structure of compound (B).



The sugar was confirmed as a glucose by paper chromatography and mixed melting point with its authentic sample and formation of its phenyl glucosazone derivative.

#### STUDY OF AGLYCONE -

A pale yellow aglycone was recrystallised from ethanol to give a colourless needles, m.p.  $224-25^{\circ}\text{C}$ , molecular formula  $\text{C}_{15}\text{H}_{12}\text{O}_5$ . Its purity was checked by paper chromatography and thin layer chromatography. The aglycone showed the following colour reactions.

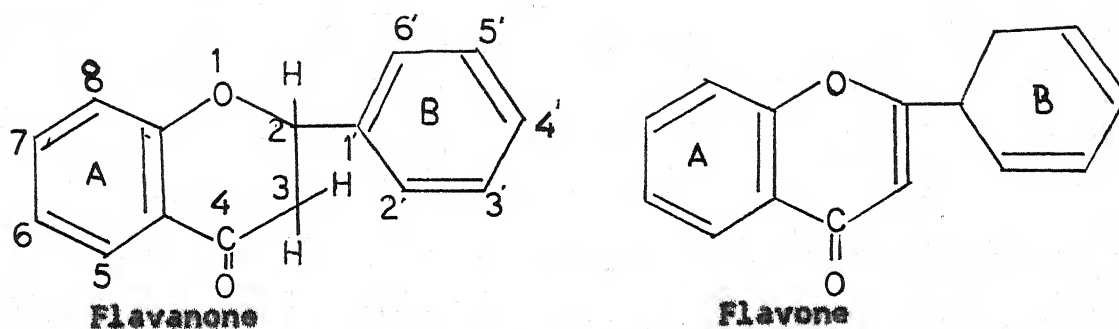
- (i) It gave deep yellow solution on decomposition with sodium hydroxide<sup>12</sup> and sodium carbonate.
- (ii) Its ethereal solution did not give any precipitate with silver nitrate and calcium chloride.
- (iii) It gave pale yellow precipitate with lead acetate.
- (iv) Its ethanolic solution gave intense violet colour with Shinoda reagent<sup>13</sup>.
- (v) It gave a yellow colour with liquid ammonia which showed fluorescence under U.V. light<sup>12</sup>.
- (vi) It gave deep green colour with alcoholic ferric chloride.<sup>14</sup>

The above colour reactions indicated the presence of flavonoid nucleus<sup>15,16</sup> in the aglycone. In addition to the above reactions the aglycone responded the following some special reactions.

- (1) It gave red colour with 2:4-dinitro phenyl hydrazine indicating the presence of ketonic group.<sup>17</sup> ( $>\text{C}=\text{O}$ ).

(11) It gave positive test with sodium boro hydride.<sup>18</sup>

From the above set of reactions it is clear that the aglycone is a flavanone derivative, not a flavone. Flavanones differ from flavones in the sense that flavanones are saturated between  $C_2$  and  $C_3$  whereas the flavones have a double bond in between  $C_2$  and  $C_3$  carbon atom.

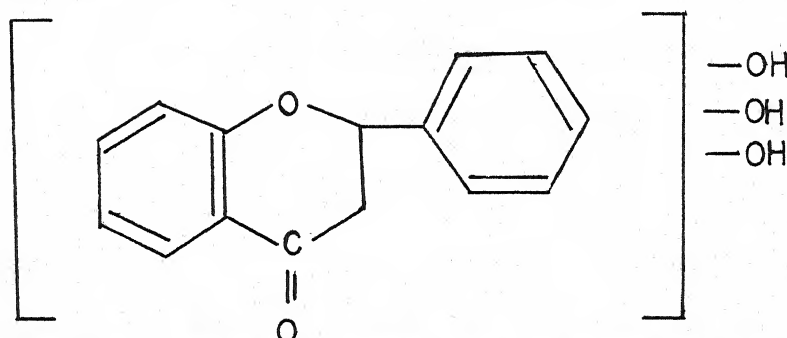


Due to the absence of double bond in between  $C_2$  and  $C_3$  in flavanones, the ring B is not in conjugation with the carbonyl group and as a result, it responded to the characteristic reactions of carbonyl group ( $>C=O$ ) and absorbed at comparatively shorter wavelength<sup>19</sup>. The absorption only in ultra violet region ( $\lambda_{max}$  270 to 290 nm)<sup>20,21</sup> also showed the presence of flavanones, in which the ring A is conjugated with ketonic group ( $>C=O$ ). But in the case of flavones, the test of ketonic functional group was not obtained and the absorption maxima in both ultra violet and visible region ( $\lambda_{max}$  240-270 nm in U. V. and  $320 - 380 \text{ cm}^{-1}$  in visible region)<sup>20,22</sup> are exhibited.

With concentrated hydrochloric acid, the aglycone gave an intense red colour which on dilution disappeared and with concentrated sulphuric acid it turned orange red solution but on warming it turned deep red, which

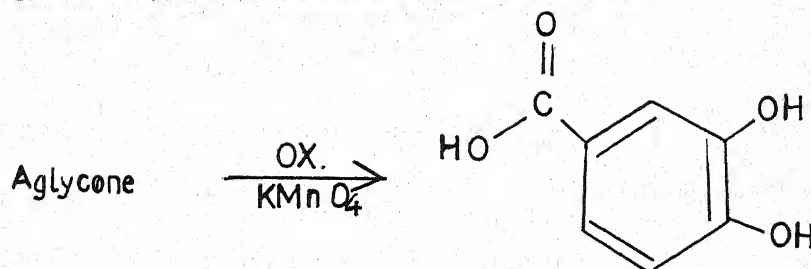
is showed that the aglycone does not contain any methoxy (O Me) or ethoxy groups.

On acetylation with acetic acid/ pyridine, aglycone gave an acetyl derivative which on analysis showed the presence of three acetyl groups. This evidently showed the presence of three hydroxyl groups in the aglycone. The presence of - OH group was further supported by the absorption peaks at  $3320\text{ cm}^{-1}$  in the I.R. spectrum. This fact was further evident from the absence of absorption peaks in the region  $2832\text{--}2815$ ,  $1190\text{ cm}^{-1}$  of the I.R. spectrum of the aglycone. Thus the basic skeleton of the aglycone can be represented as follows.



In aglycone, the position of the hydroxyl groups was confirmed at 3' and 4' in the ring B on the basis of the following reactions .

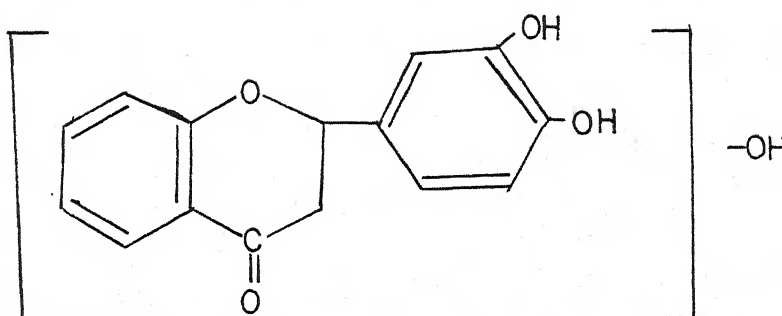
The aglycone, on oxidation with neutral potassium permanganate gave a protocatechuic acid





The identity of oxidised product was confirmed by mixed melting point, melting point and co-chromatography with its authentic sample.

Thus the structure of aglycone can be represented as further .



Some reactions were observed by aglycone which are given below.

- (i) It gave negative test on heating with fused sodium acetate and acetic anhydride followed by the addition of concentrated hydrochloric acid<sup>23</sup> (Pecheco Reaction).
- (ii) No reaction was obtained with Zn / HCl<sup>24</sup>.

These reactions indicated the absence of hydroxyl group at position - 3 .

The absence of hydroxyl group at position - 5 of the aglycone was suggested by the fact that no bathochromic shift was observed in the UV. region by the addition of a few drops of 1% ethanolic aluminium chloride solution<sup>25</sup>. In U.V. light no fluorescence was observed with ethanolic aluminium chloride<sup>26,27</sup> or zirconium oxy chloride<sup>28</sup> separately; no colour with boric acid in the presence of citric acid in acetone and imparted no fluorescence in U.V. light.<sup>29,30</sup>

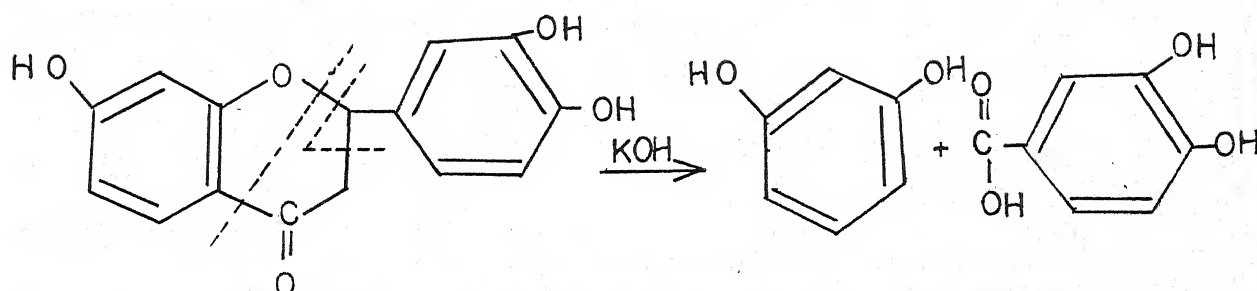


The remaining free hydroxyl group of the aglycone at position - 7 was suggested by the following reactions .

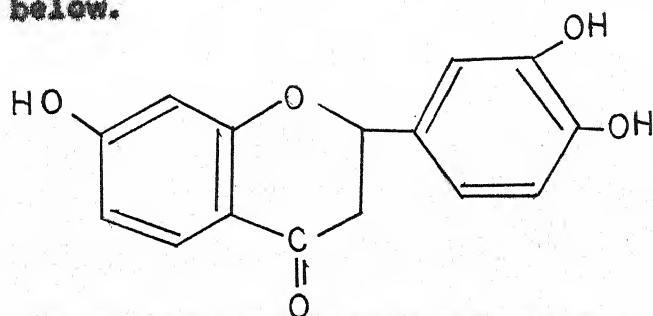
(i) A pink colour was obtained with vanillin hydro-chloric acid reagent.<sup>31</sup>

(ii) The bathochromic shift in the U.V. region by the addition of 1% ethanolic sodium acetate solution<sup>25</sup> confirmed the position of -OH group at position - 7 in ring A.

On alkaline hydrolysis with 5% potassium hydroxide , degraded products were resorcinol and protocatechuic acid (Brigg's and Locker<sup>32,33</sup>)



Thus the structure of aglycone can be represented as below.



7,3',4 - Trihydroxy flavanone.

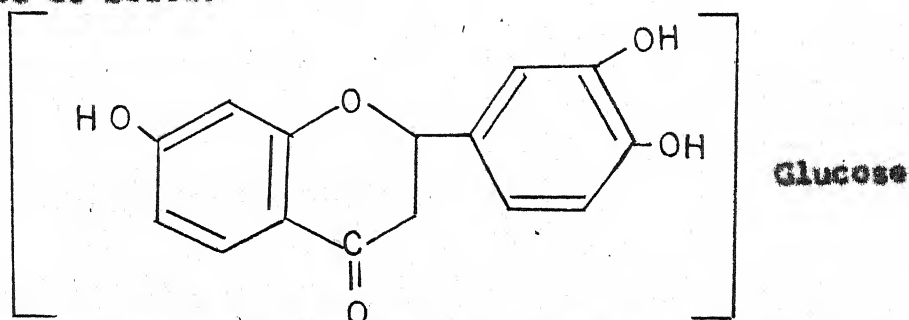
The structure of the aglycone was further confirmed by its m.m.p., co-chromatography and super impossibility of the authentic sample.

### STUDY OF SUGAR -

The hydrolysate of compound (B) was neutralised with Barium carbonate and filtered. The filtrate was concentrated to syrupy mass. This reduced Fehling's solution and gave the blue spot with aniline hydrogen phthalate. It was chromatographed on Whatmann No. 1 filter paper used n-butanol : acetic acid : water (4:1:5  $v/v/v$ ) as a solvent and sprayed with aniline hydrogen phthalate, which gave single spot, Rf value 0.18, indicated the presence of glucose. It was further confirmed by m.m.p., osazone formation and co-chromatography with its authentic sample.

### POSITION OF LINKAGE -

On the basis of the above observations the compound (B) is a flavanone glycoside which may be represented as below.

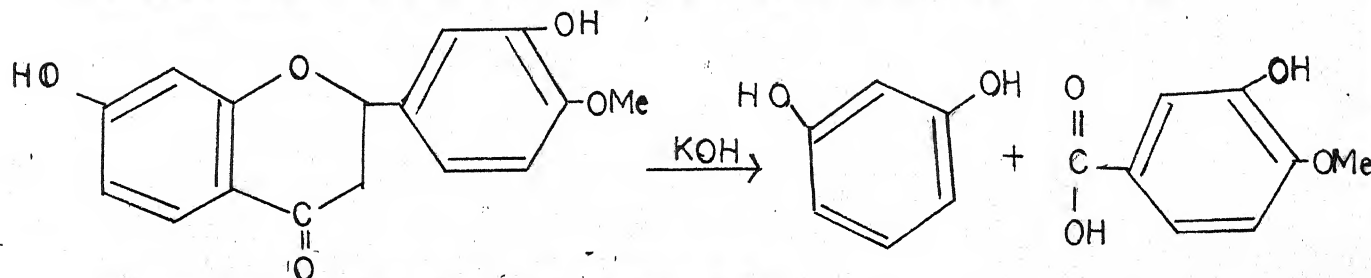


- (i) The aglycone gave a pink colour with vanillin hydrochloric acid reagent (presence of 7-hydroxy grouping in the aglycone).
- (ii) The bathochromic shift at 35 nm by the addition of sodium acetate was also showed by aglycone.

From the above reactions it is evident that the aglycone have a free hydroxyl group at position-7.

On the other hand the glycoside did not give above reactions showed that the 7-hydroxyl group is linked with the sugar moiety.

The actual position of the sugar in glycoside was deduced by complete methylation with dimethyl sulphate and potassium carbonate in dry acetone. The methylated glycoside was hydrolysed with 5% methanolic sulphuric acid to give 3', 7 - dihydroxy - 4' - methoxy flavanone which clearly indicated that the sugar moieties were attached in the glycoside at position 3' - and 7 - . The above product clearly indicated that the glycoside has a free hydroxyl group at position - 4'. The alkaline degradation of the methylated hydrolysed product also confirmed the position of sugar at position - 7 and - 3' of the ring A and B respectively.



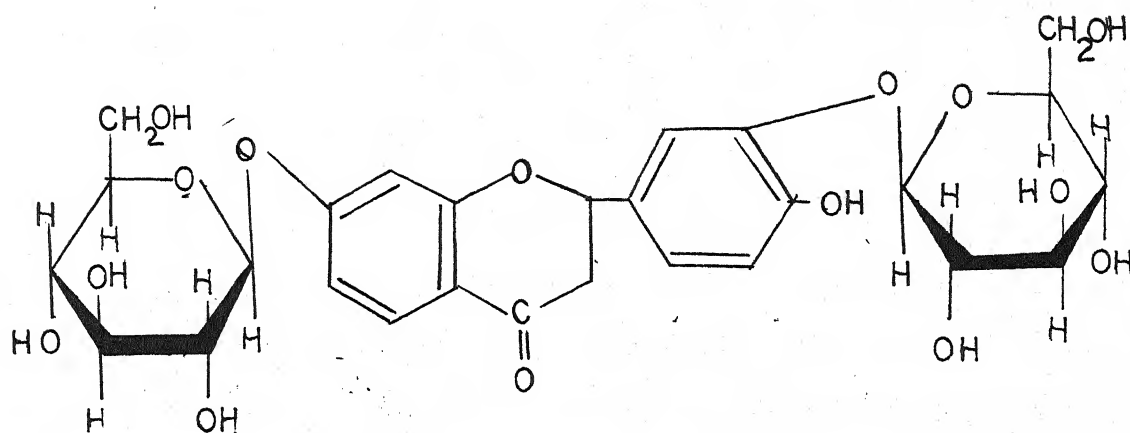
#### STUDY OF THE ORIGINAL COMPOUND (B) .

The compound (B) on per-iodate oxidation consumed 3.12 moles of periodate with the liberation of 1.16 moles of formic acid per moles of the compound (B), showing the presence of two sugar units in the compound (B). The periodate oxidation studies also showed that both the sugars were present in pyranose form.

The compound (B) was hydrolysed with emulsin enzyme<sup>34</sup> indicated  $\beta$  - linkage<sup>35,36</sup> between the sugar and the aglycone.

The actual position of the sugar units in the glycoside was deduced from the results obtained by alkaline degradation of the completely methylated glycoside.

Thus the compound (B) can be represented finally as 4'-hydroxy flavanone 7, 3'-O- $\beta$ -D-diglucopyranoside.



4'-Hydroxy flavanone 7, 3'-O- $\beta$ -D-diglucopyranoside



## EXPERIMENTAL

### 1. EXTRACTION AND ISOLATION

The compound (B) was isolated with the ethereal extract from the leaves of *Butea monosperma* as described on page 15. It was purified and recrystallised from hot distilled water and ethanol. A colourless glistening long needles crystalline compound (B), having m.p.  $190^{\circ}\text{C}$ , molecular formula  $\text{C}_{27}\text{H}_{32}\text{O}_{15} \cdot 2\text{H}_2\text{O}$  was obtained.

[Found : loss of  $\text{H}_2\text{O}$  at  $120^{\circ}\text{C}$ , 5.84, 5.79,  $\text{C}_{27}\text{H}_{32}\text{O}_{15} \cdot 2\text{H}_2\text{O}$ , requires  $\text{H}_2\text{O}$ , 5.69%. The air dried substance on combustion C, 51.24, 51.26, 51.21; H, 5.69, 5.84, 5.78; and the fully dried substance gave C, 54.32, 54.19; H, 5.67, 5.64,  $\text{C}_{27}\text{H}_{32}\text{O}_{15} \cdot 2\text{H}_2\text{O}$  requires C, 51.26; H, 5.69% and  $\text{C}_{27}\text{H}_{32}\text{O}_{15}$  requires C, 54.3% H, 5.6%]. Its homogeneity and purity were checked by thin layer chromatography.

### 2. SOLUBILITY

In cold water it was perfectly soluble to form a colourless solution. It was moderately soluble in hot ethanol, methanol and glacial acetic acid. It was readily soluble in pyridine. It was insoluble in petroleum ether, benzene, ether, chloroform, acetone, carbon tetrachloride and bromoform.

### 3. THIN LAYER CHROMATOGRAPHY

Thin layer chromatography was done on plate of silica gel-G using methanol as a developing solvent.

Single spot was obtained by exposing with iodine vapours for few minutes.

#### 4. PAPER CHROMATOGRAPHY-

Descending type of paper chromatography was done using Whatmann No.1 filter paper and the following solvent system .

(i) n - butanol : acetic acid : water ( 4:1:5 v/v ).

(ii) n - butanol : acetic acid: water ( 6:1:5 v/v ) .

By exposing with vapours of ammonia , a yellow coloured fluorescence was obtained under U V light.

$R_f$  found 0.87 in solvent system (i) and 0.48 in solvent system (ii).

#### 5. COLOUR REACTIONS.

(i) To ethanolic solution of the compound (B) (2 ml) added few drops of alcoholic ferric chloride. No colour was observed.

(ii) To ethanolic solution of compound (B) was added a pinch of magnesium and hydrochloric acid (2 ml), a deep pink colour was produced.

(iii) On whatmann No.1 filter paper , a yellow ring was obtained on exposing with ammonia vapours.

(iv) To take ethanolic solution of compound (B) (2 ml) and was added neutral lead acetate (2 ml), a yellow O-palescence was obtained.

#### 6. GLYCOSIDE NATURE OF THE COMPOUND (B)-

To take about (5 mg) of the compound (B) in ethanol, 2-3 drops of 1%  $\alpha$ -naphthol in ethanol was added followed by the addition of concentrated sulphuric acid with the sides of the test tube. A violet green ring formation at the junction of the two layers indicated the presence of sugar in the compound (B) (Molisch's test). It did not reduce Fehling's solution, Tollen's reagent and also did not obtain colour with aniline hydrogen phthalate. These reactions indicated that the sugar moiety in glycoside was not in free state.

#### 7. ACID HYDROLYSIS -

Compound (B) (100 mg) was dissolved in small quantity of 5% dilute sulphuric acid (50 ml) under reflux for 2 hours, gave a pale yellow solution, was kept for several days. A pale yellow solid mass was deposited which was separated by filtration and precipitate was washed thoroughly with distilled water. The later product on fractional crystallisation with alcohol gave a colourless needles, m.p. 224-225°C .

#### 8. IDENTIFICATION OF SUGAR MOIETY-

The aqueous hydrolysate was neutralised with Barium carbonate filtered and concentrated under reduced pressure. The hydrolysate reduced Fehling's solution and gave a spot with aniline hydrogen phthalate. It gave single spot in paper chromatography with irrigating solvent system n-butanol: acetic acid: water (4:1:5 v/v) and  $R_f$  value



was 0.18 which corresponded to glucose. The presence of glucose was confirmed by m.m.p., co-chromatography with its authentic sample and phenyl glucosazone formation, m.p.  $202^{\circ}\text{C}$ .

## 9. STUDY OF AGLYCONE -

A pale yellow coloured aglycone was recrystallised from ethanol to give a colourless needles, m.p.  $224 - 25^{\circ}\text{C}$ .

### 9.1 ELEMENTAL ANALYSIS OF AGLYCONE-

Anal., data      Found (%).      Calcd. for  $\text{C}_{15}\text{H}_{12}\text{O}_5$

C = 66.07

C = 66.27 %

H = 4.41

H = 4.40 %

### 9.2 CHROMATOGRAPHY OF AGLYCONE .

The purity of aglycone was checked by thin layer chromatography on the chromatoplates of silica gel-G using chloroform : methanol (4:1 v/v) mixture. On exposing the plates to iodine vapours, single spot was obtained.

### 9.3 COLOUR REACTIONS -

The aglycone gave all the positive colour reactions for flavanone as described on page 26 of the thesis.

### 9.4 ACETYLATION AND ACETYL PERCENTAGE DETERMINATION

To take aglycone (40 mg) in 250 ml flask



and added acetic anhydride (5.0 ml) and pyridine (3.0 ml). The whole reaction mixture was left over night. Then the reaction mixture was poured in ice cold water with constant stirring. The product was crystallised from acetone, m.p.  $202^{\circ}\text{C}$ . The percentage of the acetyl group was found to be 32.8% by the method of Wiesenberger<sup>37</sup> as described by Belcher and Godbert.<sup>38</sup>

Anal. data	found (%)	Calcd. for $\text{C}_{15}\text{H}_{19}\text{O}_5(-\text{C}(=\text{O})\text{CH}_3)_3$
C = 63.11		C = 63.31 %
H = 4.60		H = 4.52 %

#### 9.5 POTASSIUM PER MANGANATE OXIDATION.

Aglycone (50 mg) was refluxed with 10% aqueous potassium per manganate solution (25 ml) on a waterbath for four hours. The reaction mixture was cooled and excess of  $\text{MnO}_2$  was removed by adding sodium bi sulphite to it. The solution was made acidic with hydrochloric acid and extracted with solvent ether. The ethereal layer was washed with sodium bi carbonate to remove the hydrochloric acid. The sodium bi carbonate soluble portion was extracted with ether which gave a product, m.p.  $198^{\circ}\text{C}$  and was identified as protocatecholic acid by m.m.p. and co-chromatography with its authentic sample.

#### 9.6 ALKALINE DEGRADATION.

The aglycone (50 mg) was dissolved in ethanol (10 ml) and refluxed with 15 ml of 50% ethanolic  $\text{KOH}$  solution at  $100^{\circ}\text{C}$  for 10 hours followed the method of

Brigg's and Locker<sup>39</sup>. After cooling, the reaction mixture was filtered off and  $\text{CO}_2$  was passed through the filtrate and again filtered. The residue so obtained was shaken with ether. The ethereal layer on evaporation gave resorcinol (m.p., m.m.p. and CC-TLC). The filtrate on acidification with a mineral acid afforded protocatechuic acid (m.p., m.m.p. and CC-TLC ).

#### 10 METHYLATION OF COMPOUND (B) -

Compound (B) (40 mg) was methylated with 5 ml. dimethyl sulphate and 1.0 gm  $\text{K}_2\text{CO}_3$  in dry acetone (20 ml). The reaction mixture was refluxed on water bath for 20 hours. After cooling, the mixture was filtered and poured in ice cold water. A yellow mass was obtained which was recrystallised from methanol.

##### 10.1 HYDROLYSIS OF METHYLATED GLYCOSIDE -

The methylated glycoside (20 mg) was hydrolysed with 2% methanolic sulphuric acid (30 ml) on a water bath for 2 hours under reflux. The reaction mixture was cooled, filtered and concentrated under reduced pressure. The concentrated solution was poured in ice cold distilled water. The precipitate was filtered off, washed well with water and recrystallised from methanol. The filtrate was neutralised with Barium carbonate and concentrated under reduced pressure to give a light yellow coloured syrup.

##### 10.2 IDENTIFICATION OF METHYLATED SUGAR -

The syrup obtained from hydrolysis .

was chromatographed on Whatmann No. 1 filter paper using n-butanol: acetic acid: water (4:1:5 v/v) as a developing solvent system. The developed chromatogram was air dried and sprayed with aniline hydrogen phthalate and heated at 120°C for 10 minutes. Single spot was obtained. The sugar was identified by their m.m.p. and co-chromatography with authentic sample.

#### 11 ENZYMATIC<sup>33</sup> HYDROLYSIS OF COMPOUND (B) -

The compound (B) (2 mg) was dissolved in aqueous ethanol (20 ml) and to this emulsin solution (25 ml) was added and the mixture was kept for four days at room temperature. Then, the mixture was extracted <sup>with</sup> ether. The aqueous layer was concentrated and the syrup so obtained, on paper chromatography gave single spot  $R_f$  0.15 in n-butanol : acetic acid: water (4:1:5 v/v) system spraying with aniline hydrogen phthalate reagent. The mixed paper co-chromatography gave single spot. This observation indicated that the sugar is linked by  $\beta$  -linkage with aglycone.

#### 12 PER-IODATE OXIDATION .

The compound (B) (25 mg) was dissolved in 25 ml aldehyde free ethanol (90 %) and to it saturated sodium meta per-iodate solution (20 ml) in ethanol was added and made it upto 50 ml in a measuring flask. A blank was similarly prepared in another 50 ml measuring flask. After 40 hours, aliquots (5 ml) were withdrawn from both the reaction mixtures. The periodate consumed was estimated by titration against standard hypo solution and the formic acid liberated by titrating <sup>against standardised</sup>



sodium hydroxide solution according to the procedure of Jones et al<sup>36</sup>. Molecular weight of the compound B = 632 for each mole of the compound B.

Moles of per-iodate consumed = 3.12

Moles formic acid produced = 1.16

### CHEMICAL STUDY OF COMPOUND (C) -

The compound (C)  $C_{40}H_{66}O_9$  was isolated with the methanol as described on page 15 from the leaves of *Butea monosperma* as a white crystalline needles, m.p.  $180^{\circ}C$ . It was soluble in methanol, ethanol, pyridine, butanol, water and sparingly soluble in chloroform, ether and acetone.

The compound (C) having m.p.  $173^{\circ}C$ , was recrystallised thrice from hot ethyl acetate and purity was checked by thin layer chromatography as well as paper chromatography. The molecular formula of compound (C) was determined by elemental analysis and molecular weight determination was carried by mass spectrometer.

The compound (C) gave positive Molisch's test showing the presence of sugar moiety which was confirmed by NMR of the compound (C) having  $\tau$  6 and  $\tau$  8. It did not reduce Fehling's solution, Tollen's reagent and did not respond to the positive test with aniline hydrogen phthalate<sup>11</sup>. The compound (C) was hydrolysed with 10 % methanolic sulphuric acid to give an aglycone and an aqueous hydrolysate. The hydrolysate reduced Fehling's solution as well as Tollen's reagent and as responded aniline hydrogen phthalate test, showing that the reducing group present in the sugar moiety in compound (C) is not free and involved in the glycosidic linkage.

The exact nature of the glycoside was detected by separate chemical examination of the aglycone and sugar moiety.

The sugar was identified by paper chromatography in n-butanol : acetic acid : water (4:1:5 v/v) system which showed single spot with  $R_f$  0.28, suggesting the presence of xylose. It was further confirmed by osazone formation and co-chromatography with its authentic sample.

#### STUDY OF AGLYCONE .

The white coloured aglycone was crystallised from ethyl acetate having m.p.  $194-96^{\circ}\text{C}$  and molecular formula  $\text{C}_{30}\text{H}_{50}\text{O}$ . The purity of the aglycone was checked by thin layer chromatography and paper chromatography. The following colour reactions were given by the aglycone of the compound (C).

- (i) A pale yellow colour was obtained by Lieber mann Burchard reaction<sup>40</sup>.
- (ii) The aglycone gave an orange red colour with few drops of thionyl chloride (Noller's test)<sup>41</sup>.
- (iii) On treatment with sulphuric acid, the chloroform solution of the aglycone gave a yellow colour which changed to deep red. (Salkowski Reaction)<sup>42</sup>.
- (iv) The chloroform solution of the aglycone with tetra-nitromethane and chloroform (1:1 v/v), gave a yellow colour (Rázicka reaction)<sup>43</sup>.
- (v) The ethanolic solution (2 ml) of the aglycone gave violet colour with 2:6- di-tert- butyl- p. Cresol in ethanol ( Brieskorn test)<sup>44</sup>.



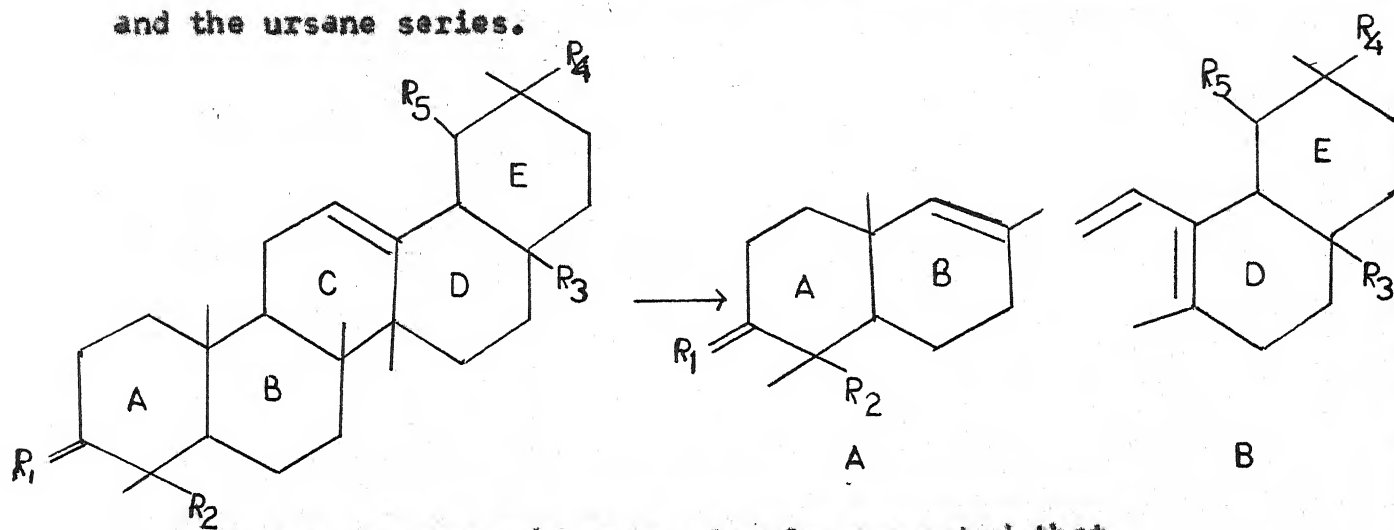
The molecular formula of the aglycone was confirmed by the molecular ion peak at  $m/e$  426 in its mass spectrum.

The colour reactions of the aglycone indicated that it is triterpenoid having pentacyclic nature.

The appearance of pale yellow colour in Liebermann Burchard reaction<sup>40</sup> and orange red colour with Noller's reagent indicated that the aglycone had an unsaturated triterpenoid part. In UV spectrum no absorption band above 212 nm, showed the absence of conjugated double bond with keto group or other double bond. The pentacyclic nature of the aglycone indicated the presence of sterically hindered double bond as shown no reduction by Adam's Catalyst<sup>45</sup>. The absorption band at 205 nm ( $\log \epsilon$  3.8) in the UV spectrum supported the presence of double bond in the aglycone molecule. The trisubstituted nature of double bond<sup>46,47,48</sup> was also evidenced by absorption band at 205 nm ( $\epsilon$  3.8). The characteristic trisubstituted linkage between the position -12 and 13 represented by the IR absorption peaks at 1653, 828 and 818  $\text{cm}^{-1}$ . The above facts were further supported by mass spectra peaks at  $m/e$  218 (a) and 203 (b) of the aglycone.

Djerassi et al<sup>49,50</sup> had established that under reverse Diel's Alder fragmentation the molecular ion of the aglycone provided a characteristic ion B containing ring D and E. The peak is generally followed by second peak corresponding to ring B minus the  $\text{C}_{17}$  substituent  $\text{R}_3$ .

The retro - Diels - Alder fragmentation is a characteristic for identifying the presence of double bond at position 12 and 13 in the triterpene of the oleanone and the ursane series.



The 218 and 203 m/e peaks clearly suggested that

- (i) Only one double bond is present in between  $C_{12}$  and  $C_{13}$ .
- (ii) No substitution in the ring C, D and E.
- (iii) The oxygen atom is either in ring A or in ring B.

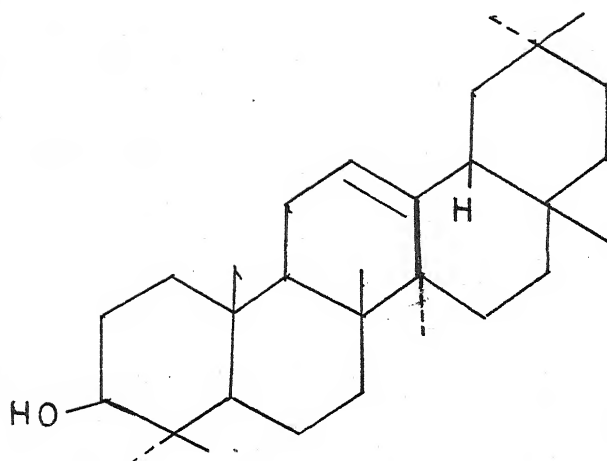
The absorption bands at 3200 , 1300 and 1100  $\text{cm}^{-1}$  in infra red spectrum are further suggested the presence of secondary hydroxyl group in the aglycone. The mono acetate  $\text{C}_{32}\text{H}_{52}\text{O}_2$  , m.p. 238-40°C and one proton signal for 3- $\alpha\text{H}$  centred at 3.28 (triplet)<sup>51</sup> and also supported the presence of one secondary hydroxyl group in the aglycone . The oxidising product , which is obtained by potassium per manganate gave positive Zimmermann test<sup>52</sup> for 3 -keto group. It showed the presence of secondary hydroxyl group which is situated at position -3 in ring A. Thus it is in close confirmity with the observation that most of the known

number of the pentacyclic triterpenes are oxygenated at  $C_3$  usually as alcohols and the configuration of the natural product is generally  $\beta$ . The  $\beta$  - nature of the hydroxyl group, present at position - 3, was further confirmed by the I R peaks at 1039 and 1000  $\text{cm}^{-1}$ , these are characteristic for the  $\beta$  -hydroxy group at position 3<sup>53</sup>. A triplet like multiplet centred at  $\tau$  6.72 in NMR spectrum for 3 $\alpha$  -proton, clearly suggested the equatorial ( $\beta$  -orientation)<sup>54,55</sup> nature of the secondary hydroxyl group at position-3. On acetylation, it formed a monoacetate  $C_{32}H_{52}O_2$ , m.p. 237-40 $^{\circ}$ C and it was not hydrolysed by methanolic sodium bicarbonate at room temperature suggesting the  $\beta$  -orientation of the hydroxyl group.

The NMR spectra in general showed olefinic proton as singlet at  $\tau$  4.9 and 8-methyl groups were observed in the range  $\tau$  9.2 to 8.45. The alcoholic group at  $C_3$  was observed at  $\tau$  6.85. The secondary alcoholic group is observed at  $\tau$  4.76 as sharp singlet and olefinic proton was exhibited as triplet centred at 4.85  
(J Cps = 6 Hz )

From the foregoing facts it is clear that aglycone belongs to the eleanane series having a double bond at  $C_{12}$  and  $C_{13}$  and an equatorial hydroxyl group at position - 3. Thus the structure of the aglycone may be represented as follows.





Olean-12-en-3- $\beta$ -ol.

The identity of the aglycone is further confirmed by mixed melting point, co-chromatography, comparative spectral studies <sup>52,56</sup> and degradative products <sup>57,58</sup> of the aglycone.

The principal fragmentation peaks of the mass spectra at  $M^+$   $m/e$  426, 411, 278, 207, 205, 203, 189, 149, 133 ( $m/e$  203 base peak) confirmed the above structure <sup>49,56</sup> of the aglycone. The fragmentation of the aglycone by mass spectral data, can be explained as follows.

#### STUDY OF SUGAR -

The hydrolysate of the compound (C) obtained on acid hydrolysis, was neutralised with Barium carbonate which was filtered, concentrated and tested for the presence of free sugar as xylose. The identity of the sugar was confirmed by paper chromatography, mixed melting point and super imposition of I R spectrum with an authentic sample. ( $R_f = 0.28$ ).

### POSITION OF LINKAGE -

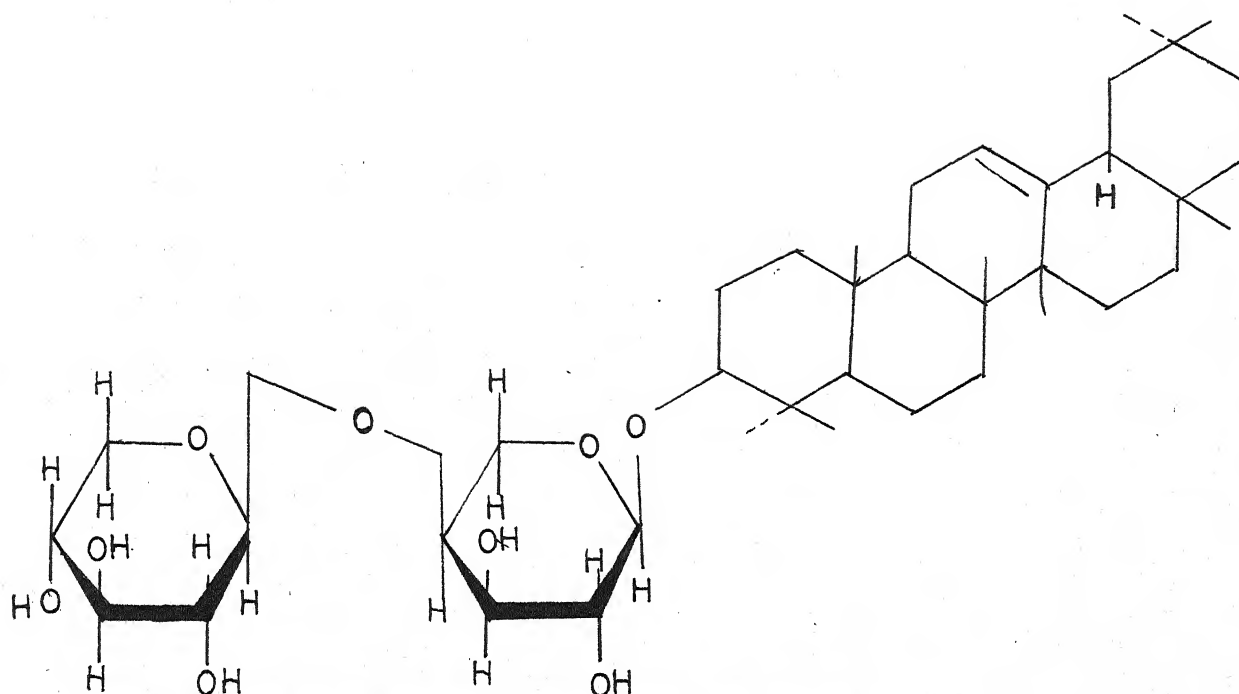
The structure of aglycone itself indicated that the position - 3 has only free hydroxyl group with  $\beta$  -orientation . Thus the linkage between xylose and aglycone is at position - 3 of the ring A . The periodate oxidation studies of the glycoside showed the consumption of 3.14 molecules of periodate with the liberation of 1.2 molecules of formic acid per molecules of glycoside. It suggested that only two unit of xylose are present in the molecule. The periodate oxidation studies also showed that both the sugars are present in pyranose form and are linked through  $C_1 \longrightarrow C_4$  linkages in the disaccharide. The glycoside on partial hydrolysis with 2% sulphuric acid and on examination of different intervals by paper chromatography, showed only xylose sugar which clearly indicated that the two xylose units are present in the sugar moiety.

The completely methylated glycoside on acid hydrolysis gave two molecules of 2,3-di-O-methyl-  $\beta$  -D-xylose. Thus two xylose units are involved in the glycosidic linkage.

### ENZYMATIC HYDROLYSIS -

On enzymatic hydrolysis<sup>59</sup> with emulsin , the glycoside referred the presence of  $\beta$  -linkage between the sugar and the aglycone and the  $\alpha$  -linkage between the two xylose units. The presence of  $\alpha$  -linkage between the xylose units were confirmed by hydrolysis of disaccharide

with maltase.

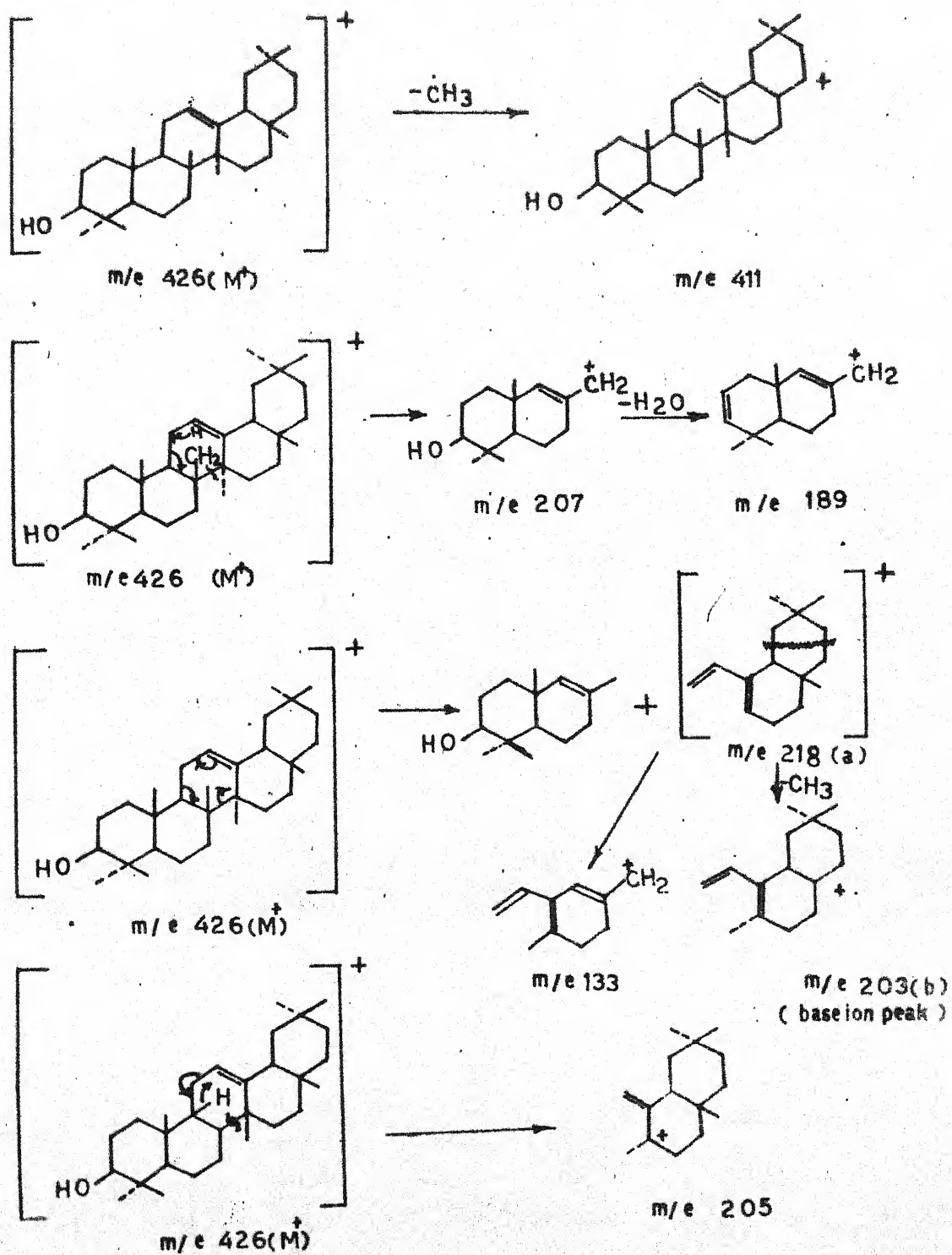


Olean-12-en-3-β-D-xyloxyloside

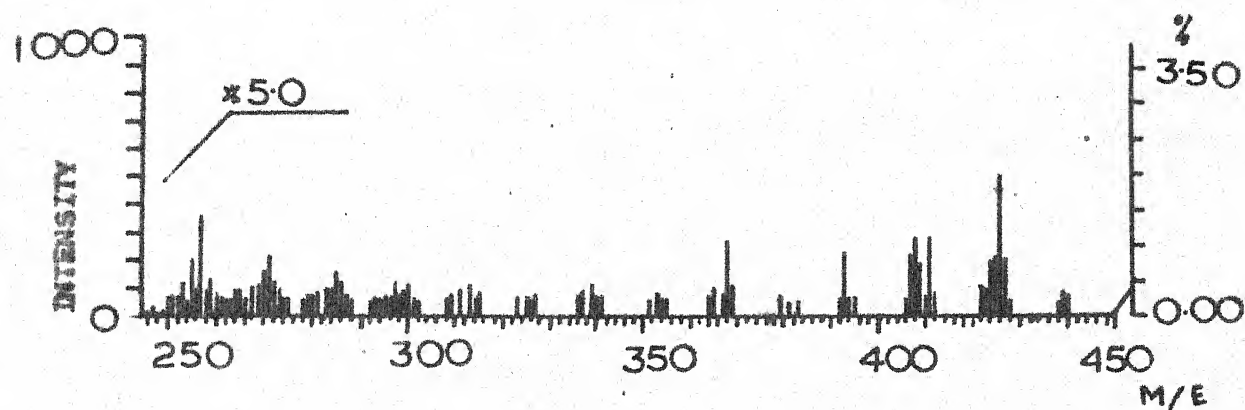
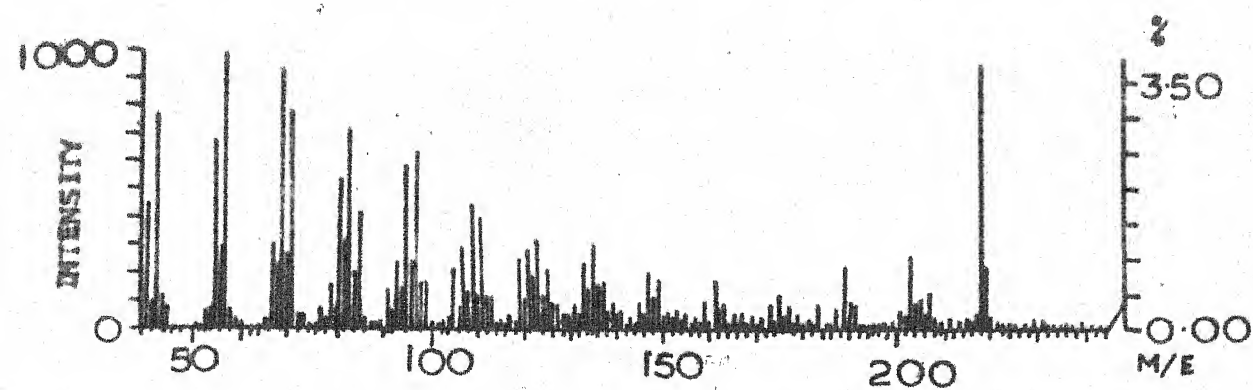
(β-Amyrin-3-xyloxyloside.)



# MASS FRAGMENTATION PATTERN OF AGLYCONE OF COMPOUND-C



# MASS SPECTRUM OF AGLYCON OF COMPOUND -C

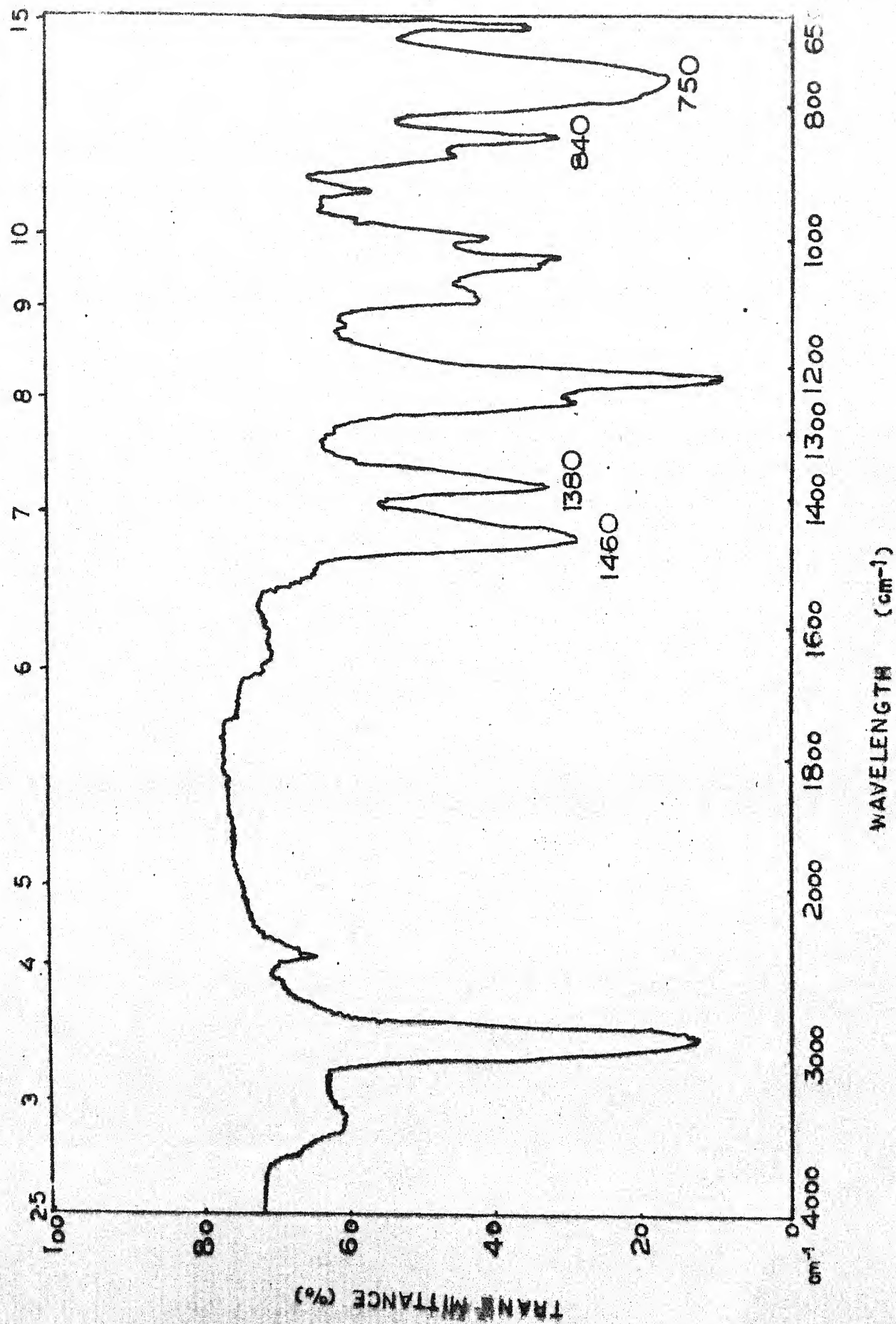


MASS SPECTRUM : ( 3 TO 4 )

BASE PEAK : M/E 57.0 INT. 21.0

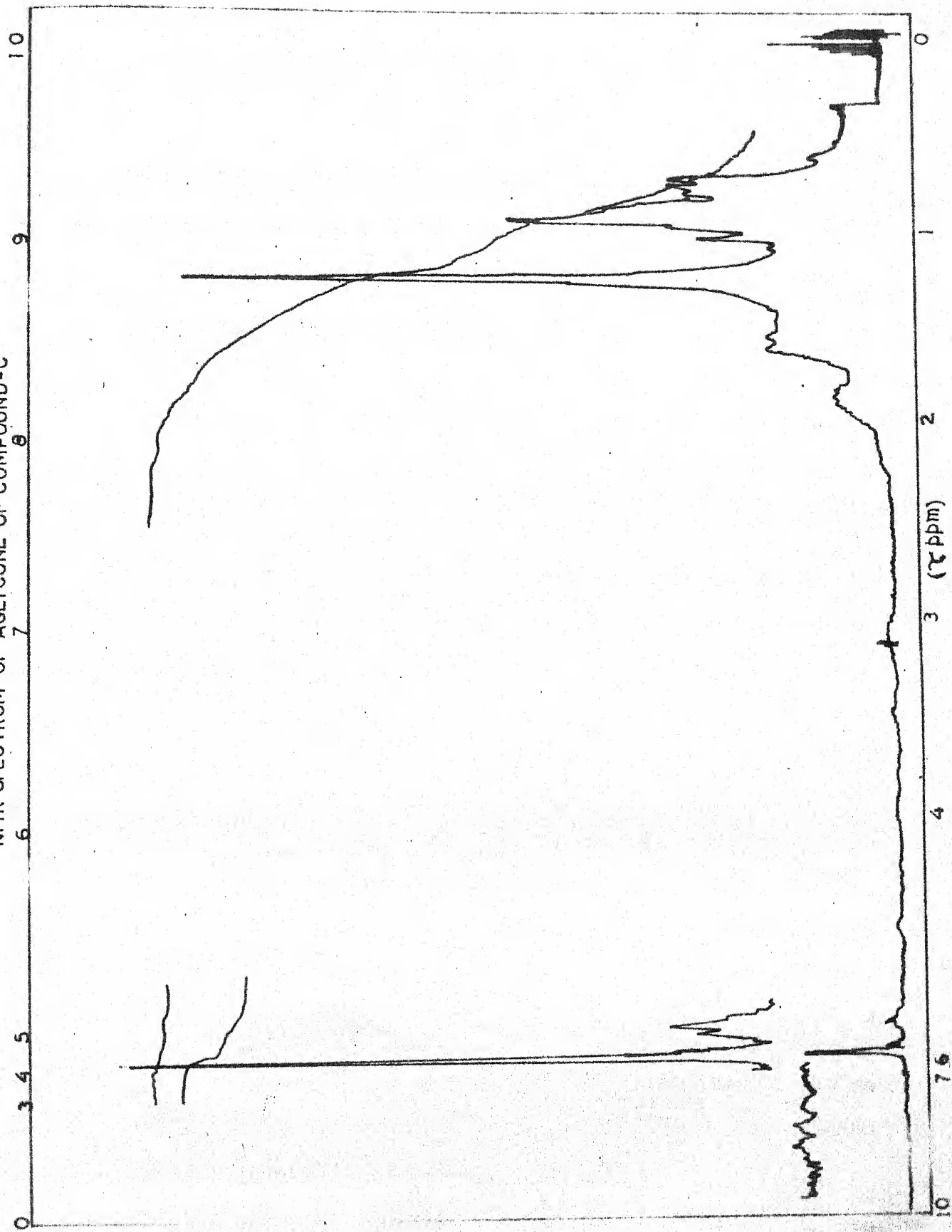
M/E	RAW INT.	R. INT.	SIGMA (%)	
257.0	4.5	72.3	16.50	
368.0	1.1	54.9	12.54	
393.0	1.0	47.7	10.89	
407.0	0.9	46.3	10.56	
408.0	1.1	54.9	12.54	
411.0	1.2	59.3	13.53	
426.0	2.1	102.7	23.43	END

INFRA RED (KBr) SPECTRUM OF AGLYCONE OF COMPOUND - C ( MICRONS )





NMR SPECTRUM OF AGLYCONE OF COMPOUND -C



## EXPERIMENTAL

### 1. EXTRACTION AND ISOLATION -

The compound (C) is obtained from the methyl alcohol extract of the ethanolic extract from the leaves of *Butea monosperma* as described on page 15 . It was purified and recrystallised from hot ethyl acetate . The compound (C) has molecular formula  $C_{40}H_{66}O_9$  and m.p.  $173^{\circ}C$  .

Its purity and homogeneity was checked over silica gel - G chromatoplates.

The compound (C) was soluble in methanol , ethanol, pyridine, butanol, water and sparingly soluble in chloroform, ether and acetone.

### 2. THIN LAYER CHROMATOGRAPHY -

The purity of the compound (C) was checked by silica gel - G chromatoplate using n- butanol : acetic acid : water (4:1:5 v/v) as a developing solvent. The developed chromatoplate of silica gel - G was sprayed with 50 % sulphuric acid and place in an oven at  $100^{\circ}C$ , a blue coloured single spot was observed.

### 3. PAPER CHROMATOGRAPHY -

Descending paper chromatography was done on Whatmann No.1 filter paper using the following solvent system-

- (1) n-butanol:acetic acid : water (4:1:5 v/v).
- (ii) Iso-butanol: acetic acid : water (5:1:4 v/v).

The spot was developed by ammonia and iodine which gave single spot.

#### 4. GLYCOSIDIC NATURE OF COMPOUND (C) -

The glycosidic nature of compound (C) was tested by Molisch's test. To take compound (C) (5 mg.) in ethanol, 3 or 4 drops of 1% ethanolic  $\alpha$  - naphthol solution was added followed by the addition of few drops of concentrated sulphuric acid with the side of the test tube. At the junction of the two layers, a violet coloured ring was formed which indicated the presence of sugar. It neither reduced Fehling solution nor gave any colour with aniline hydrogen phthalate<sup>11</sup>. These informations indicated that the reducing group in sugar moiety is not free but involved in glycosidic linkage.

#### 5. ACID HYDROLYSIS.

The compound (C) (200 mg.) was hydrolysed with 10 % methanolic sulphuric acid for 10 hours on a water bath. After cooling, the contents were diluted with water and shaken with chloroform. The ethereal layer was separated out. After evaporation of the solvent, a white precipitate was obtained as an aglycone which was crystallised from ethyl acetate.

#### 6. IDENTIFICATION OF SUGAR MOIETY -

The aqueous hydrolysate was neutralised with Barium carbonate and filtered. The filtrate was reduced Fehling solution and gave a dark blue colour



with aniline hydrogen phthalate. A filtrate was concentrated and chromatographed, which gave single spot,  $R_f$  0.28 in n-butanol : acetic acid : water (4:1:5 v/v). The sugar was identified as xylose. It was confirmed by co-chromatography, m.m.p. and super imposition with its authentic sample.

## 7. STUDY OF AGLYCONE -

The aglycone, obtained from ethereal layer, was crystallised from hot ethyl acetate, into white needles, having m.p.  $194 - 196^\circ\text{C}$   $[\alpha]_D^{25} + 34.0^\circ$ , highly soluble in pyridine, hot ethanol and methanol.

### 7.1 COLOUR REACTIONS OF AGLYCONE -

#### (i) Liebermann Burchard Reaction<sup>40</sup> -

The aglycone (5 mg.) was dissolved in chloroform (2ml) and to it 5 drops of acetic anhydride were added followed by the addition of 2 drops of concentrated sulphuric acid, where upon a pale yellow colour was obtained.

#### (ii) Neller Test<sup>41</sup> -

The aglycone gave an orange red colour with a few drops of thionyl chloride (prepared by adding 0.01 % stannic chloride in pure thionyl chloride).

#### (iii) Salkowski Reaction<sup>42</sup> -

The chloroform solution of the aglycone on treatment with concentrated sulphuric acid, gave a yellow colour which changed to deep red.

(iv) Ruzicka Reaction<sup>43</sup> -

The aglycone was dissolved in chloroform and to this tetranitro methane in chloroform (1:1 v/v) was added, a yellow colour was obtained.

(v) Brieskorn Test<sup>44</sup> -

The aglycone (2 mg.) in ethanol (2 ml) was treated with 2:6-di tert. butyl-p-cresol in ethanol, a violet colour was obtained.

(vi) Zimmermann Test<sup>52</sup>.

The oxidised product (2 mg.) was dissolved in 1 ml. of 2 N potassium hydroxide in absolute ethanol. After 15 minutes, the mixture was diluted to 10 ml. with absolute ethanol. A violet colour developed which faded after some time.

7.2 ELEMENTAL ANALYSIS -

Anal. Data found (%)

C = 84.49

H = 11.74

Calcd. for  $C_{30}H_{50}O$

C = 84.50 %

H = 11.72 %

By mass spectrum .

7.3(A) ACETYLATION OF THE AGLYCONE -

The aglycone (50 mg.) was acetylated with acetic anhydride (5 ml.) and pyridine (5 ml.) at room temperature for keeping about 48 hours. The acetylated mixture was poured into cold water. The precipitate was filtered and crystallised from chloroform : methanol mixture to yield an acetylated derivative molecular formula  $C_{32}H_{52}O_2$  . m.p. 238-40°C.,

$[\alpha]_D^{25} + 80.0^\circ$ .

### 7.3 (B) ELEMENTAL ANALYSIS OF ACETYL DERIVATIVE-

Anal. data found (%)	Calcd. for $C_{32}H_{52}O_2$
C = 82.10	C = 82.06 %
H = 11.13	H = 11.12 %

### 7.4 OXIDATION OF THE AGLYCONE-

Aglycone (50 mg.) was refluxed with 25 ml of 1 % alcoholic potassium permanganate solution for about four hours. The reaction mixture was cooled. The excess of  $MnO_2$  was neutralised by the addition of sodium bi sulphide. It was acidified with dilute hydrochloric acid and extracted with ether. The ethereal layer was washed with sodium bi carbonate. The sodium bi carbonate soluble portion on neutralisation and extraction with ether gave a product having m.p.  $201^{\circ}C$ . The oxidised product was identified as a keto compound which was confirmed by Zimmermann test<sup>52</sup>.

### 7.5 (A) METHYLATION OF AGLYCONE-

The aglycone (40 mg) was taken into a round bottom flask with dry acetone (20 ml) dimethyl sulphate (5 ml) and anhydrous potassium carbonate (1 gm). The whole reaction mixture was refluxed on water bath for 24 hours. It was cooled and poured into ice cold water. A yellow mass was settled down which was filtered and recrystallised from methanol.

### 7.5 (B) DETERMINATION OF METHOXYL PERCENTAGE-

The methoxyl percentage in methylated aglycone was determined by the method of Balcher, Fildes and Nutton<sup>60</sup>.



Anal. data found (%)	Calcd. for $C_{30}H_{49}O(OCH_3)$
Percentage of methoxyl group	Percentage of methoxyl group
= 6.90 %	= 6.79 %

### 8. IDENTIFICATION OF HYDROLYSATE -

The hydrolysate, obtained during hydrolysis, was examined by paper chromatography using *n*-butanol : acetic acid : water (4 : 1 : 5 v/v) as developing solvent. After drying the paper was sprayed with aniline hydrogen phthalate and kept in an oven at 120°C about 10 minutes which gave single spot,  $R_f$  value 0.28, which was identified as xylose. Further, it was confirmed by co-chromatography, m.m.p. and super imposition of I R with its authentic sample and osazone derivative.

### 9. METHYLATION OF COMPOUND-(C) .

The compound (C) was methylated as described on page 54. The methylated derivative was crystallised from methanol.

#### 9.1 HYDROLYSIS OF METHYLATED COMPOUND-(C)-

The methylated glycoside (20 mg.) was hydrolysed with 2 N-methanolic sulphuric acid (20 ml.) on a water bath for 4 hours. The hydrolysed product was cooled, concentrated and poured in distilled water. The precipitate was filtered and washed well with water. It was crystallised from methanol. The filtrate was neutralised with Barium carbonate and again filtered and concentrated under reduced pressure. The filtrate was a

light yellow coloured syrup. It was hygroscopic in nature.

## 9.2 IDENTIFICATION OF METHYLATED SUGAR -

The methylated sugar was chromatographed on Whatmann No. 1 filter paper using n-butanol: acetic acid: water (4 : 1 : 5 v/v) as a developing solvent. The chromatogram was air dried and sprayed with aniline hydrogen phthalate and heated at 100°C for 10 minutes, single spot was obtained. The  $R_{\text{TMG}}$  (TMG = 2,3,4,6-tetra-o-methyl-D-glucose) value of the spot was found to be 0.76 which corresponded to 2,3-di-o-methyl-D-xylose respectively. The identity of the sugar was confirmed by co-chromatography with its authentic sample.

## 10. PARTIAL HYDROLYSIS OF COMPOUND -(C)-

The compound (C) (100 mg.) was hydrolysed by refluxing with 2 % methanolic sulphuric acid and hydrolysate was examined at different intervals by paper chromatography. After two and half an hour xylose could be detected.

## 11. HYDROLYSIS OF THE COMPOUND (C) WITH FORMIC ACID -

The compound (C) (200 mg.) was dissolved in boiling cyclohexanol (10 ml.) and hydrolysed formic acid (7% 8 ml.) by refluxing on a waterbath for half-an hour. The aqueous hydrolysate gave Molisch's test and not gave any prompt test of monosaccharide but on further hydrolysis with 7% ethanolic sulphuric acid gave xylose only.

## 12. PERIODATE OXIDATION OF COMPOUND (C).

The glycoside (50 mg.) was dissolved in a mixture of ethanol (50 ml.) and distilled water (50 ml.) and .5 M sodium metaperiodate (50 ml.) was added to it. The solution was made up to 250 ml. with ethanol and allowed to stand for 48 hours. The periodate consumed and formic acid was liberated which were estimated by the titrimetric method of Jones et al.<sup>61</sup>. The results are as follows.

Molecular weight of the compound (C) = 690

For 1.5 ml aliquots of the reaction mixture

0.01 N sodium hydroxide consumed = .60 ml.

0.01 hypo consumed = 3.2 ml.

For each mole of glycoside

Moles of formic acid liberated = 1.2

Moles of periodate consumed = 3.14

## 13. ENZYMIC HYDROLYSIS.

Emulsin used for hydrolysis of the glycoside, was extracted from bitter almonds by the following method.<sup>62</sup>

The almonds were put in boiling water for one minute and then the brown skin, which has been loosened, was removed. The almonds were crushed in a mortar in small pieces. Distilled water (30 ml.) was added to it to make a paste. 10% acetic acid (10 ml.) was added to it and it was mixed well and was allowed to stand for 5 minutes. Stirring was done at different intervals, then it was filtered through Buchner funnel and the residue in the funnel was thoroughly washed with distilled



water. Then one drop of 10% acetic acid was added to the filtrate till the clear solution became turbid, therefore, a little more of acetic acid was added drop by drop, until no more precipitate was formed. Then it was refiltered. The clear filtrate was used for hydrolysis.

To take compound (C) in distilled water (10 ml.) was added 5 drops of toluene and emulsin (50 mg.). Put this reaction mixture at room temperature for 72 hours. Then, the mixture was extracted with ethyl acetate. The aqueous layer was concentrated under reduced pressure. The concentrate did not reduce Fehling's solution and it gave positive Molisch's test, indicated that the sugar was attached with aglycone in the  $\beta$  -linkage and on paper chromatography with n-butanol : acetic acid: water (4 : 1 : 5 v/v) spraying reagent aniline hydrogen phthalate did not give any spot and both the sugar units are attached by  $\alpha$  -linkage.

The disaccharide was hydrolysed by maltase enzyme which is specific for  $\alpha$  -linkage.

#### 14. ABSORPTION SPECTRUM -

(i) UV spectrum was recorded on Beckman model DU spectrophotometer.

(a) GLYCOSIDE -  $\lambda$  max: 205 nm ( $\log \epsilon$  3.8).

(b) AGLYCONE -  $\lambda$  max: 207 nm

#### (11) I R SPECTRAL DATA (KBr).

The following prominent peaks ( $\text{cm}^{-1}$ ) were observed in the I R spectrum of the aglycone.

Position of the absorption band (in $\text{cm}^{-1}$ )	Assignment
3200 (sh)	C-H Stretch vibration <sup>50</sup>
2900 (s)	C-H stretching in methyl/
2817 (w, Shoulder)	Of $\text{CH}_2$ <sup>63</sup> .
1470 (s)	C - H bending in methyl/ or $\text{CH}_2$ <sup>63</sup> .
1380 (sh, doublet)	$\text{C}(\text{CH}_3)_2$ gem dimethyl group
1355	resulting from symmetric and asymmetric -C - $\text{CH}_3$ vibration (Triterpen type) <sup>64,65</sup> .
1110 (sh)	-C-O stretching of secondary alcohol. <sup>66</sup>
1300 (w)	Secondary OH bending vibration. <sup>66</sup>
918 (w)	= C-H bending ) Trisubstituted vibration } Olefinic type <sup>52,66</sup> .
828 (m)	
1653 (w, shoulder)	C=C stretching vibration
1200 (w)	C-C (Stretching vibration) <sup>67</sup>
1435 (w, shoulder)	Adjacent - $\text{CH}_2$ to ethylenic double bond <sup>52</sup>
1340 (w)	C-H bending vibration
1039 (a)	C-O stretching ) Due to the vibration } hydroxyl at 69 position - 3
1027 (a)	
1000 (a)	C-C stretching ) or due to vibration } C-C stretching or cyclohexane skelton <sup>67</sup> .

(iii) NMR SPECTRAL DATA ( $\tau$  ppm) ( $\text{CDCl}_3$ )

---

Aglycone -  $\tau$  9.92 - 8.45 (m, methyl and methylene group);

$\tau$  4.85 (t,  $J = 6\text{Hz}$ , H, Olefinic ) and

$\tau$  4.76 (s, 1 H,  $\text{C}_3\text{-OH}$ )

(iv) MASS SPECTRAL DATA .

---

Aglycone -

In the mass spectrum of the aglycone following prominent peaks were observed.

m/e 426 ( $\text{M}^+$ ), 411, 218, 207, 205, 203 (base peak),  
189, 149, 133.



### CHEMICAL STUDY OF COMPOUND - (D)

A brownish red coloured long prismatic compound (D) molecular formula  $C_{27}H_{30}O_{15} \cdot H_2O$ , m.p.  $199-200^{\circ}C$  (decomposed), isolated with ethyl alcohol extract from the leaves of *Butea monosperma*. It gave  $R_f$  value 0.66 in n-butanol : acetic acid : water (4 : 1 : 5 v/v) when sprayed with ammonia.

The compound (D) was recrystallised from butyl alcohol. Its homogeneity was checked by thin layer chromatography and paper chromatography using solvent n-butanol : acetic acid : water. Its molecular formula was determined by cryoscopic method as well as elemental analysis.

It gave positive Molisch's test but neither reduced Fehling's solution nor gave any colour with aniline hydrogen phthalate, showing the presence of glycoside.

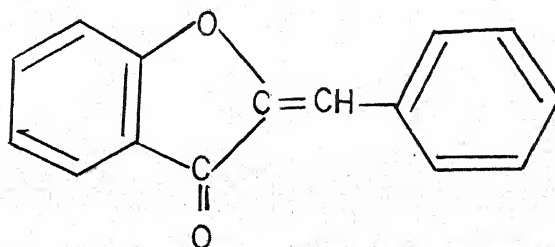
The ethanolic solution of the compound (D) gave the following colour reactions.

- (i) It produced deep red colour with aqueous sodium hydroxide solution<sup>70</sup>.
- (ii) It gave no colour reaction when treated with magnesium and hydrochloric acid<sup>70,71</sup>.
- (iii) It gave pale yellow colour when treated with sodium-analgun followed by an acid.<sup>70</sup>

- (iv) It gave red colour when treated with a few drops of concentrated sulphuric acid.<sup>70,71.</sup>
- (v) It gave deep red colour when treated with few drops of concentrated nitric acid.
- (vi) It gave an orange colour with sodium carbonate solution.
- (vii) It gave green colour when treated with ethanolic ferric chloridesolution.

Most of the reactions given above are for the flavanoid compounds. However, red colour with concentrated sulphuric acid and no colour with magnesium and hydrochloric acid suggested that the compound (D) — should be an aurone rather than flavone etc. This conclusion is further supported by the fact that the compound (D) showed strong absorption maxima at 330 nm and 425 nm<sup>72</sup> (visible region).

Thus the compound (D) should have the following skeleton.



The acid hydrolysis of the compound (D) with 7% aqueous alcoholic sulphuric acid gave an aglycone having molecular formula  $C_{15}H_{10}O_5$  and an aqueous hydrolysate. The hydrolysate reduced both, the Fehling's solution and the Tollen's reagent and it also

gave a brown colour with aniline hydrogen phthalate<sup>11</sup>, showing thereby that the reducing group in the sugar moiety present in the compound (D) is not free and hence is involved in the glycosidic linkage. Thus the compound (D) is supported to be a glycoside.

The exact nature of the compound (D) was detected by separate chemical examination of the aglycone as well as the sugar moiety was confirmed as a glucose by paper chromatography, m.m.p. with its authentic sample.

#### STUDY OF AGLYCONE -

A deep orange yellow prismatic aglycone crystallised from absolute ethanol, having the molecular formula  $C_{15}H_{10}O_5$ , m.p.  $310-12^{\circ}C$  (decomposed), showing the following colour reactions.

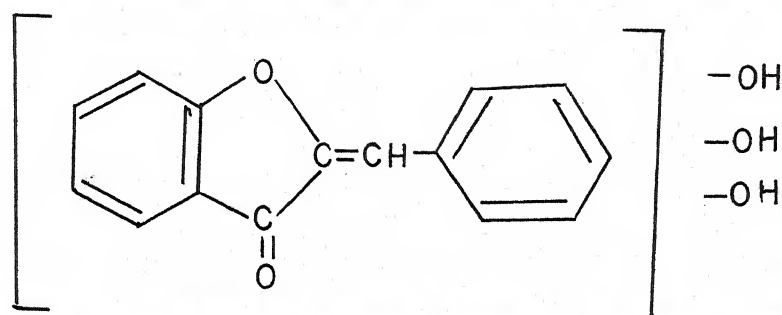
- (i) It gave an olive brown colour with alcoholic ferric chloride.
- (ii) It gave red colour with concentrated sulphuric acid .
- (iii) It gave purple red colour with aqueous sodium hydroxide.
- (iv) It did not respond to Shinoda reduction.
- (v) It produced purple red colour when sprayed with ammonia.

The above positive reactions indicated the presence of auren.

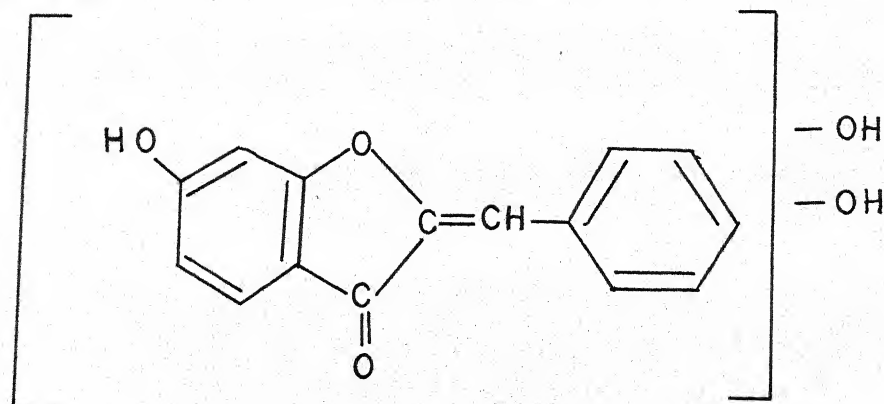


The aglycone on treatment with acetic anhydride and pyridine and methylation with dimethyl sulphate and potassium carbonate in boiled acetone formed triacetylated and trimethylated products respectively showing the presence of three hydroxyl groups in the aglycone. The aglycone neither gave any peak in Infra red spectrum nor gave the presence of methoxyl group by Ziesel's method<sup>73</sup> of methoxy group estimation.

Thus on the basis of the above facts the structure of aglycone can be represented as follows.

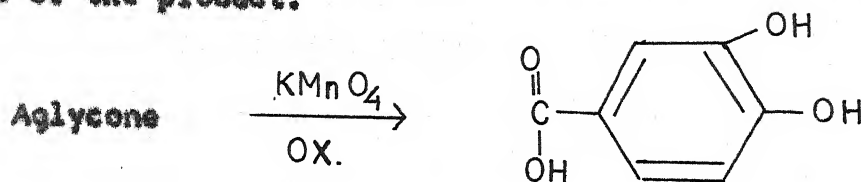


Since the aglycone has  $\lambda_{\text{max}}$  at 318 nm which showed that 6-hydroxy group is free in aglycone molecule as it has been noted that the presence of hydroxyl group in the ring A para to carbonyl group causes a large hypsochromic shift in the aurone series<sup>74</sup>. Thus the structure of aglycone may be represented now as follows.



This aglycone on treatment with alkaline solution under vigorous condition yielded, resorcinol which showed that no other group is attached with ring A .

An aglycone was oxidised with neutral potassium permanganate, yielded protocatechuic acid as one of the product.

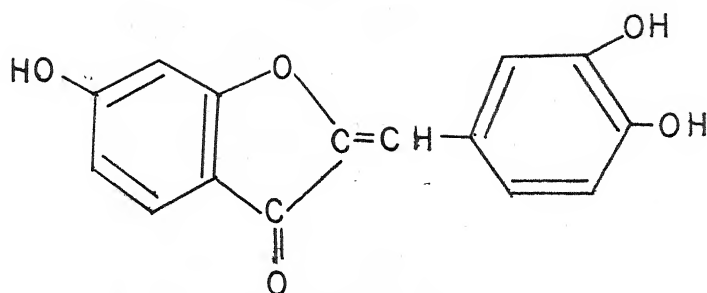


Protocatechuic acid.

This suggested that ring B contains two free hydroxyl groups at position- 3' and 4' . Finally the presence of 3' - and 4' - di hydroxyl groups confirmed by absorption maxima at 365 nm and 375 nm in addition of sodium ethoxide and anhydrous aluminium chloride in ethanolic solution of the aglycone respectively.

The structure of aglycone was confirmed as 2-(3,4-dihydroxy benzylidene )-6-hydroxy coumaran-3-one by comparison with a sample prepared by its synthesis.

From the above facts the structure of aglycone may be as follows.



3' , 4' , 6 - trihydroxy aurone

Or  
2 -(3' , 4' - dihydroxy Benzyldene)-6-  
hydroxycoumaran - 3 - one .

#### STUDY OF SUGAR -

The sugar was identified as glucose by co-chromatography, mixed melting point with an authentic sample and osazone formation. The periodate oxidation gave two molecules of glucose per molecule of glycoside.

#### POSITION OF LINKAGE -

The position at which sugar moiety is linked to the aglycone, has to be decided and from the structure of aglycone it is obvious that it may be either of three 6, 3', 4' - positions. Another point to be decided is whether the glycosidic linkage is  $\alpha$  - or  $\beta$  - .

#### STUDY OF ORIGINAL COMPOUND -(D).

Since the glycoside on potassium permanganate oxidation did not give protocatechuic acid as one of the product. The complete methylation of the glycoside followed by hydrolysis , gave a mono-methyl ether having



m.p.  $241-42^{\circ}\text{C}$  (I R  $2850\text{ cm}^{-1}$ ) showing that one of the glucose unit attached with  $3^{\text{rd}}$  - position.

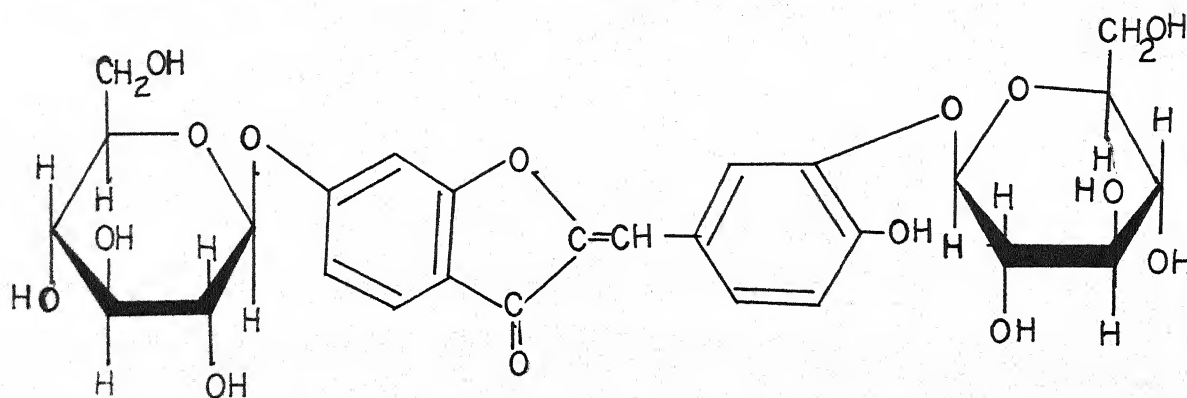
The above facts indicated that the another molecule of the glucose unit is attached with position -6 of the ring A.

Since the glycoside was hydrolysed with emulsin showing the  $\beta$  -linkage at both the positions of glucose units.

The per iodate oxidation studies and I R peak at  $845\text{ cm}^{-1}$  also showed that both the sugar units are in the pyranose form.

Finally , the structure of the compound (D) was confirmed by its synthesised product.

On the basis of the above facts the structure of the glycoside was elucidated as below.



4' - hydroxy - Aureone - 6, 3' -O -  $\beta$  - D- di-glucose-  
pyranoside.

## EXPERIMENTAL

### 1. EXTRACTION AND ISOLATION -

The compound (D) was isolated with ethandiac extracts from the leaves of *Butea monosperma* as described on page 15 . It was purified and recrystallised from butyl alcohol having molecular formula  $C_{27}H_{30}O_{15} \cdot H_2O$ , m.p.  $199-200^{\circ}C$ ,  $M^+$  612.

The homogeneity of the compound (D) was checked by thin layer chromatography using silica gel- G as adsorbent and n-butanol : acetic acid : water (4 : 1 : 5 v/v) as solvent. The compound (D) was soluble in dioxan, pyridine, butyl alcohol, ethyl alcohol and water.

### 2. ELEMENTAL ANALYSIS -

Anal. data found (%)	Calcd. for $C_{27}H_{30}O_{15} \cdot H_2O$
C = 53.3	C = 52.9 %
H = 5.60	H = 5.2 %
H <sub>2</sub> O = 2.9	H <sub>2</sub> O = 3.0 %

### 3. PAPER CHROMATOGRAPHY -

Descending paper chromatography was done on Whatmann No.1 filter paper using the solvent.

- (i) Phenol : water (9:1 v/v)
- (ii) n- butanol : acetic acid : water (4:1:5 v/v)
- (iii) n-butanol : acetic acid : water ( 5:1:5 v/v)

Single spot was obtained when it was exposed with ammonia .

#### 4. COLOUR REACTIONS OF COMPOUND (D) .

All the colour reactions which were given by the compound (D) was described on page 61 of the thesis.

#### 5. GLYCOSIDIC NATURE OF THE COMPOUND (D)

To take compound (D) (5 mg) in ethanol and 2 to 3 drops of 1 % solution of  $\alpha$ -naphthol in chloroform, in a boiling tube, was added few drops of concentrated sulphuric acid with the side of the test tube. A violet ring was formed at the junction of the two layers, indicated the positive Molisch's test. The compound (D) neither reduced Fehling's solution nor gave any test with aniline hydrogen phthalate. These tests represented the absence of free reducing group in the glycosidic linkage.

#### 6. ACID HYDROLYSIS -

To take compound (D) (100 mg) and 7 % aqueous alcoholic sulphuric acid (25 ml) in a 250 ml round bottomed flask. The whole reaction mixture was refluxed on water bath for 2 hours, cooled and filtered. An orange yellow solid was obtained which was washed thoroughly with distilled water. It was recrystallised from ethyl alcohol as deep orange yellow prisms, m.p.  $310-12^{\circ}\text{C}$  (decomp.) The filtrate was finally extracted with ether to remove the last traces of aglycone. Further, it was neutralised with Barium Carbonate, filtered and concentrated under reduced pressure to a syrupy mass.



## 7. STUDY OF AGLYCONE-

A deep orange yellow coloured aglycone, having molecular formula  $C_{15}H_{10}O_5$ ,  $M^+$  270, m.p.  $310-12^\circ C$  (Decomp.) was crystallised from absolute ethanol. It was soluble in dioxan, butanol, pyridine, ethanol, methanol and water.

### 7.1 COLOUR REACTIONS OF AGLYCONE.

The aglycone gave all positive colour reactions for an aurone as described on page        of the thesis.

### 7.2 PAPER CHROMATOGRAPHY OF AGLYCONE .

The purity of aglycone was checked by paper chromatography on Whatmann No.1 filter paper. Single spot was obtained by using different solvent system.

(i) Phenol : water ( 9 : 1 v/v)

(ii) n-butanol : acetic acid : water (4:1:5 v/v)

(iii) n-butanol : acetic acid : water (5:1:5 v/v).

### 7.3 ELEMENTAL ANALYSIS OF AGLYCONE.

Anal. data found (%)

C = 67.0

H = 4.0

Calcd. for  $C_{15}H_{10}O_5$

C = 66.7 %

H = 3.7 %

### 7.4 METHYLATION OF AGLYCONE

The aglycone (20 mg) was methylated with dimethyl sulphate (7.5 ml.) and  $K_2CO_3$  (0.5 gm.) in dry acetone (50 ml). The reaction mixture was refluxed on water bath for 20 hours. After refluxing the reaction mixture was cooled, filtered and poured in ice cold water, where upon a yellowish mass was settled down. It was filtered and crystallised from dilute alcohol as colourless

rectangular prisms and plates, m.p.  $184-186^{\circ}\text{C}$ .

#### 7.5 DETERMINATION OF METHOXYL PERCENTAGE-

By Belcher, Fildes and Nutten method<sup>60</sup> the percentage of the methoxyl group in the methylated aglycone was obtained.

Anal. data found (%)	Calcd. for $\text{C}_{15}\text{H}_7\text{O}_2(\text{COCH}_3)_3$
Methoxyl group = 30.0	Methoxyl group = 29.80 %
C = 69.2	C = 69.20 %
H = 5.05	H = 5.10 %

It was identified by its mixed melting point and co-chromatography with an authentic sample which was obtained from Central Drug Research Institute, Lucknow.

#### 8. IDENTIFICATION OF SUGAR -

The syrupy solution was obtained by the hydrolysis of the glycoside. It was examined on paper chromatography using the solvent phenol : water (9:1 v/v). The developed chromatogram was air dried and sprayed with aniline hydrogen phthalate and on heating at  $110^{\circ}\text{C}$  for 10 minutes, single spot of  $R_f$  value 0.60 was obtained, which corresponded to glucose.

The sugar was identified by mixed melting point and co-chromatography of the authentic sample.

#### 9. DETERMINATION OF SUGAR AND AGLYCONE IN THE GLYCOSIDE.

Anal. data found (%)	Calcd. for $\text{C}_{27}\text{H}_{30}\text{O}_{15}\cdot\text{H}_2\text{O}$
Glucose = 58.5	Glucose(2moles) = 58.8%
Aglycone = 44.4	Aglycone = 44.1%

## 10. METHYLATION OF COMPOUND-(D)

The compound (D) (0.5 gm.) was refluxed in dry acetone (100 ml.) with dimethyl sulphate (1.5 ml.) and  $K_2CO_3$  (3 gm.) for 30 hours on a water bath. After methylation the acetone was distilled off, cooled and water was added to dissolve the salt. Enough sulphuric acid was added to neutralise  $K_2CO_3$ . Then the whole reaction mixture was filtered off.

### 10.1. HYDROLYSIS OF METHYLATED COMPOUND -(D).

The methylated solution was made acidic with dilute sulphuric acid. The mixture was boiled for four hours, cooled and diluted with distilled water. It was extracted with ether. After evaporation of the solvent, yellow solid was obtained which was crystallised from ethanol as orange yellow prismatic needles, having m.p.  $241-242^\circ C$ . Its identity was confirmed by mixed melting point and co-chromatography with its synthesised product.

### 10 .2. ELEMENTAL ANALYSIS OF METHYL ETHER

Anal. data found (%)

Calcd. for  $C_{16}H_{12}O_5 \cdot 2H_2O$

C = 60.5

C = 60.0 %

H = 4.5

H = 5.0 %

$H_2O$  = 10.5

$H_2O$  = 11.2 %



The sample was dried at 120°C

Anal. data found (%)

Calcd. for  $C_{16}H_{12}O_5$

OMe = 10.7

O Me = 10.9 %

### 10.3. IDENTIFICATION OF METHYLATED SUGAR

The filtrate, which was obtained after the separation of aglycone, was neutralised with  $BaCO_3$ . It was filtered and concentrated to a syrupy mass. It was chromatographed on Whatmann No.1 filter paper using phenol : water (9:1 v/v), n-butanol : acetic acid : water (4:1:5 v/v) as a developing solvent. The air dried developed chromatogram was sprayed with aniline hydrogen phthalate at 120°C for 10 minutes. Single spot was obtained. The TMG value (TMG = 2,3,4,6-tetra-O-methyl-D-glucose) of the spot was found to be 1.00 which corresponded to 2:3:4:6-tetra-O-methyl-D-glucose. The sugar was confirmed by co-chromatography with the authentic sample, obtained from Sugar Institute Kanpur.

### 11. PERIODATE OXIDATION OF COMPOUND -(D)

Compound D (.25 mg.) was dissolved in ethanol (25 ml) and added distilled water (25 ml) in a conical flask. Sodium meta periodate (.25 ml.) was also added to it. The solution was made upto 100 ml. with ethanol

and allowed to stand for 48 hours . The consumed periodate and liberated formic acid were estimated by titrimetric method of Jones et al . 61.

The results are given below -

Molecular weight of the compound (D)	= 612
For each mole of the glycoside	-
Moles of formic acid liberated	= 1.2
Moles of periodate consumed	= 3.16

## 12. ENZYMIC HYDROLYSIS -

Compound (D) (20 mg.) was dissolved in aqueous ethanol ( 20 ml.) and to this , emulsin solution (25 ml.) was added. The whole reaction mixture was kept for four days. Then, the reaction mixture was extracted with solvent ether. The aqueous layer was concentrated to syrupy mass, which on paper chromatography gave single spot  $R_f$  0.19 in n- butanol : acetic acid : water (4:1:5 v/v ) system spraying with aniline hydrogen phthalate reagent. Mixed paper and co-chromatography gave single spot. The ethereal layer gave the test of aglycone as described earlier.

### SYNTHESIS OF AGLYCONE TRIMETHYL ETHER.

The synthesis of trimethyl ether was completed in two steps.

#### (a) Synthesis of MONO METHYL ETHER - 6-hydroxy-2-(4-hydroxy -3-methoxy benzyldene)- coumaran -3-one

To take (2 gm.) solution of 6-hydroxy coumaran-3-one<sup>75</sup> and 4 gm. vanillin solution in 20 ml. ethanol was treated with 30 ml. of aqueous KOH (30 mg. in 35 ml.). The reaction mixture was kept at room temperature for three days with occasional shaking. Diluted with water and acidified with HCl. The mono methyl ether was obtained which was crystallised from dilute alcohol as an yellow rectangular prisms, m.p. 263-64°C.

#### (b) METHYLATION-

When mono methyl ether was heated in acetone with dimethyl sulphate and  $K_2CO_3$  for 12 hours, trimethyl ether was obtained which was crystallised from ethyl alcohol twice and yielded rectangular prisms and plates having m.p. 184-186°C.

The degradation product, spectral studies were similar to those observed in the case of trimethyl ether obtained from the methylation of the glycoside.

### SYNTHESIS OF MONOMETHYL ETHER -

It was described above.



SYNTHESIS OF TRIHYDROXY AGLYCONE -

2 - (3:4-dihydroxy benzylidene) 6-hydroxy coumaran-3-one.

The solution of 6-hydroxy coumaran-3-one (1.50 gm.) and protocatechualdehyde (1.38 gm.) in alcohol (20 ml.) was saturated with HCl (ice cooling) in 2 hours. This mixture was diluted with water and extracted with ether. The solvent on evaporation gave a solid which on crystallisation from ethyl alcohol gave orange yellow prisms having m.p.  $310-12^{\circ}\text{C}$ . Its identity was confirmed by the hydrolysis of the glycoside i.e. compound (D) .

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CHAPTER - III

CHEMICAL EXAMINATION OF RHIZOME  
OF CURCUMA LONGA

The plant *Curcuma longa*, commonly known as Haldi or Turmeric, belongs to the family Zingiberaceae. It is a house hold remedy found all over India, China and Pakistan.

The plant *Curcuma longa* is an important spice which is used all over the world. It is a very important medicinal spice for domestic purposes. It is a tall herb having large root stock which is oval in shape and are tubers and orange coloured inside. The leaves are very large having blade like petiole which is oblong lanceolate and tapering to the base. The flowers are autumnal spikes 4-6 inch long.

It is known to be of great medicinal importance.<sup>1</sup> The rhizome is a house hold remedy for many diseases. It has many important medicinal values. It is an aromatic stimulant tonic and it provides a relief in catarrhal and cough. The rhizome of turmeric is also given in diarrhoea, intermittent fever, dropsy, jaundice, liver disorders and urinary diseases. The fresh juice of turmeric is given in bronchitis. In weak state of digestion and flatulent, the turmeric is given in twice a day.

It is used as an excellent dressing, sprains bruises, black eyes, inflammatory infections of the joints. It is also an efficacious remedy for catarrhal and purulent ophthalmia. Its lotion or paste is applied to small pox and chicken pox eruption, itching, eczema



and other parasitic skin diseases. Inhalation of fumes of burning Turmeric is also an important remedy for nasal catarrh and hysteric fits.

CONSTITUENTS	PART OF THE PLANT	REFERENCES
(i) Essential oil, Oleoresin, Curcumin	Rhizome	Trop. Sci., <u>19</u> (1), 37-45(1976)(Eng.)
(ii) Curcumin	Rhizome	Varasarn Passacharsathara, <u>8</u> (2), 29-31(1981)(Eng.)
(iii) $\alpha$ - and $\beta$ -Turnerene	Rhizome	J. Chem. Soc., Chem. Commun., <u>6</u> , 363-4(1982)(Eng.)
(iv) Curcumin	Rhizome	J. Pharm. and Pharmacol., <u>5</u> , 448-57(1953); Current Sci., <u>21</u> , 311-12(1952)
(v) Essential oil, $\alpha$ -phellandrene-1, borneol, zingerene and sesquiterpene(Turmerones).	Rhizome	Indian Inst. Sci., <u>17 A</u> , 7-24(1933).
(vi) Curcuminoid	Rhizome	J. Chem. Soc., Perkin Trans., <u>1</u> , 2379-88(1973)(Eng.)
(vii) Essential oil, an orange yellow pigment, arabinose, fructose and glucose.	Rhizome	Sci. Res., <u>4</u> (4), 193-4(1967)(Eng.)
(viii) Campesterol, Stigmasterol, $\beta$ -sitosterol, Cholesterol and fatty acids(satd. straight chain, satd. iso-, monoenoic and dienoic acids).	Rhizome	(Cole. Pharm. Seoul. Natl. Univ. Seoul, S. Korean) Seoul Eachehkye Yakhak. Non-Munjip, <u>1</u> , 105-11(1976)(Korean)

The literature survey of the proposed plant revealed that the little work has been done on the rhizome of the *Curcuma longa*. The essential oils and colouring materials are reported from this part of the plant. Therefore, it is worthwhile to explore the studies to get some more important medicinal constituents of the plant. The study of Curcumin has been done in detail in the present Chapter of this thesis.

# EXTRACTION AND ISOLATION FROM THE RHIZOME OF CURCUMA LONGA

The dried and crushed rhizome of the plant *Curcuma longa* was defatted with petroleum ether (60-80°C) in a Soxhlet extractor for 48 hours. The petroleum ether extract was concentrated to a small volume under reduced pressure. It was chromatographed by column chromatography using silica gel-G as adsorbent and petroleum ether as an eluent. The effluent of ether, on concentration gave a single compound (E), which was shown by thin layer chromatography and paper chromatography.

The defatted residue was extracted with absolute ethanol and concentrated to a syrupy mass. It was refluxed with petroleum ether and benzene for complete defatation. The remaining extract was dissolved in ethyl acetate and was chromatographed by a column chromatography using silica gel -G as an adsorbent and benzene : ethyl acetate (1:1 v/v) as an eluent. This effluent on concentration gave an orange yellow shining crystalline compound (F) .



## RHIZOME OF CURCUMA LONGA

Defatted with Petroleum ether (60-80°C)  
in Soxhlet extractor

Extract

Concentrated under  
reduced pressure and  
passed under column .

Compound (E)

Defatted material

Extracted with ethanol

Extract

washed with Benzene

Residue

Filtrate

Residue

Soluble in ethyl  
acetate and passed  
through silica gel-G  
column

Compound (F)

### CHEMICAL STUDY OF COMPOUND (E)

An orange yellow compound(E) ,having molecular formula  $C_{15}H_{20}O$  , B.P.  $280-282^{\circ}C$  , isolated with the rhizome of the *Curcuma longa* as described on page 86 . It was soluble in petroleum ether, benzene, ethyl acetate, chloroform, carbon tetra chloride acetone, methanol, ethanol and pyridine.

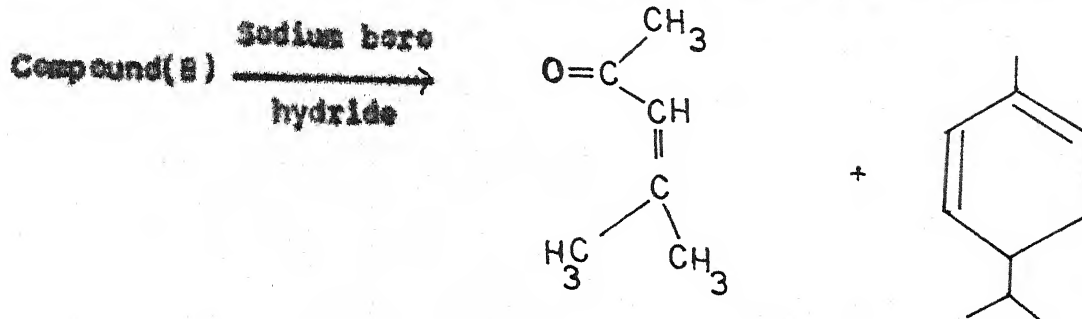
The compound(E) gave the following colour reactions :-

- (i) It gave red colour with 2:4-di nitro phenylhydrazine.
- (ii) It reduced Fehling's solution and Tollen's reagent.
- (iii) It decolourised bromine water and potassium permanganate solution showing the presence of unsaturation.
- (iv) It gave positive tri nitro methanetest indicating the presence of olefinic bond in the compound (E) (Ruzicka test<sup>2</sup>).

The compound (E) gave  $M^{+}$  216 as base peak in Mass spectrum, I.R. Peaks 3050 and 3025, 1638(  $>C=O$  ), 1622 (  $>C=CH-$  ), 1517 and 821  $cm^{-1}$ . The above spectral data showed that the compound (E) contains One olefinic double bond and one ketonic group. Thus , it may be  $\alpha$  monocyclic aromatic sesquiterpene.

As compound (E) could not be acetylated and methylated indicating the absence of any free hydroxyl group. The colour reaction No. 1 also evidenced the presence of ketonic group in the compound (E) .

The presence of  $-C-CH_3$  Group in the compound(E) was determined by reduction with sodium boro hydride, it gave mesityl oxide and,  $\alpha$ -phellandrene as its products.



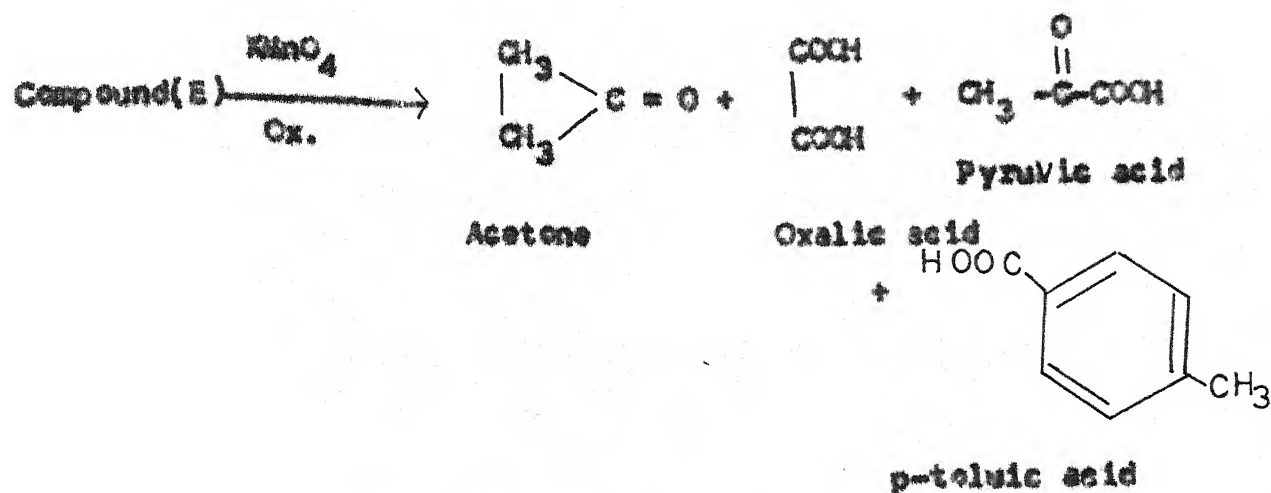
The compound(E) showed the characteristic absorption bands at  $1685\text{ cm}^{-1}$  ( $\gamma > C=O$ ) and  $1620\text{ cm}^{-1}$  ( $\gamma > C=C <$ ),  $1515(-C_6H_4-)$  and  $819\text{ cm}^{-1}$  for  $(pCH_3-C_6H_4)$ . The N.M.R. ( $CHCl_3$ ) is showed 4-aromatic protons as a singlet centered at  $\tau$  3.03, the signal at  $\tau$  4.09 (m) exhibited olefinic protons attached to isopropylidene group.

Two methyl groups of isopropylidene group showed geometrical isomerism as shown signal of methyl groups. Methyl group which cis to carbonyl group is absorbed at  $\tau$  7.02 as singlet. The methyl group trans to carbonyl group are exhibited as doublet at  $\tau$  8.19 due to long range coupling of H attached to olefinic bond. The  $\alpha$ -methylene protons are absorbed at  $\tau$  7.90 as multiplet. The tertiary methyl proton was shown as multiplet centered at  $\tau$  4.10 and two methyl groups were exhibited at  $\tau$  8.80 as doublet. The mass spectrum showed base peak  $m/e$  216.

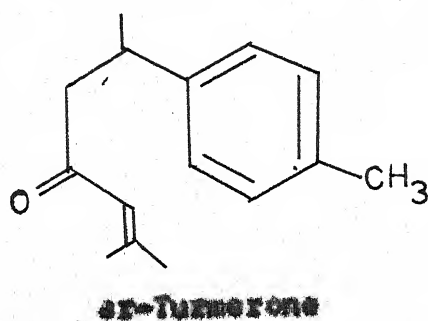
On oxidation with potassium permanganate solution, the compound (E) gave different products as depicted



below.



From the foregoing discussion it is obvious that the structure of compound (E) may be as follows:-



This structure of compound (E) was confirmed by m.m.p., co-chromatography and super imposition of I R spectrum with its authentic sample.

## EXPERIMENTAL

### 1. EXTRACTION AND ISOLATION

The compound (E) was isolated with the petroleum ether extract by column chromatography using silica gel-G as an adsorbent as described on page 86. It had molecular formula  $C_{15}H_{20}O$ ,  $M^+$  216, B.P. 280-292°C. The homogeneity of the compound (E) was checked on the chromatoplate of silica gel-G using petroleum ether (40-60°C).

### 2. ELEMENTAL ANALYSIS

Anal. data found(%)	Calcd. for $C_{15}H_{20}O$
C = 83.42	C = 83.29 %
H = 9.38	H = 9.32 %
$M^+$ 216	$M^+$ 216

### 3. SOLUBILITY

The compound (E) was soluble in most of the Organic solvents.

### 4. COLOUR REACTIONS OF COMPOUND (E)

All the colour reactions of the compound (E) was described on page 88 of the thesis.

### 5. HYDROGENATION OF COMPOUND (E)

The hydrogenation of the compound (E) (50 mg.) was carried out by ethanolic sodium boro hydride (0.5 mg.), to yield hydrogenated product according to the report in literature.

## 6. POTASSIUM PERMANGANATE OXIDATION OF COMPOUND(E)

The mixture of compound (E) (50 mg.) and 10 % aqueous potassium permanganate solution (5 ml.) were refluxed in flask (250 ml) on a water bath for four hours. The mixture was cooled and excess of  $\text{MnO}_2$  was removed by addition of  $\text{NaHSO}_3$ . The later solution was acidified with dilute hydrochloric acid (10 ml) and extracted with solvent ether. The ethereal extract after washing thrice with sodium bi sulphite was concentrated under reduced pressure to give an oily mass which was chromatographed using silica-gel-G as adsorbent and methanol as eluent to afford different products. The products were confirmed by their elemental analysis, m.m.p. and co-chromatography with their authentic samples.

## 7. ABSORPTION SPECTRAL DATA OF COMPOUND (E)

### 7.1 U.V. SPECTRAL DATA (nm)

$\lambda_{\text{max}}(\text{CH}_3\text{OH})$  237 and 263 nm

### 7.2. I.R. SPECTRAL DATA (KBr) ( $\text{cm}^{-1}$ )

The prominent peaks of compound(E) were at 1685, 1620, 1515 and 819  $\text{cm}^{-1}$ .

### 7.3. N.M.R. SPECTRAL DATA ( $\text{CHCl}_3$ )

$\tau$  3.03, 4.09, 4.10, 7.02, 7.50, 8.19

and 8.80.

### 7.4. MASS SPECTRAL DATA

m/e 216( $\text{M}^+$ ) 201, 132, 119, 105, 98, 91,

83, 77 and 55.



### CHEMICAL STUDY OF COMPOUND (F).

An orange yellow compound (F) , having molecular formula  $C_{21}H_{20}O_6$  ,  $M^+$  368 , m.p. 184-85°C was isolated with rhizome of *Curcuma longa* as described on page . It was recrystallised from methanol. It was soluble in petroleum ether (40-60°C) , benzene, ethyl acetate, chloroform, acetone , methanol and ethanol.

The ethanolic solution of the compound (F) gave following colour reactions :-

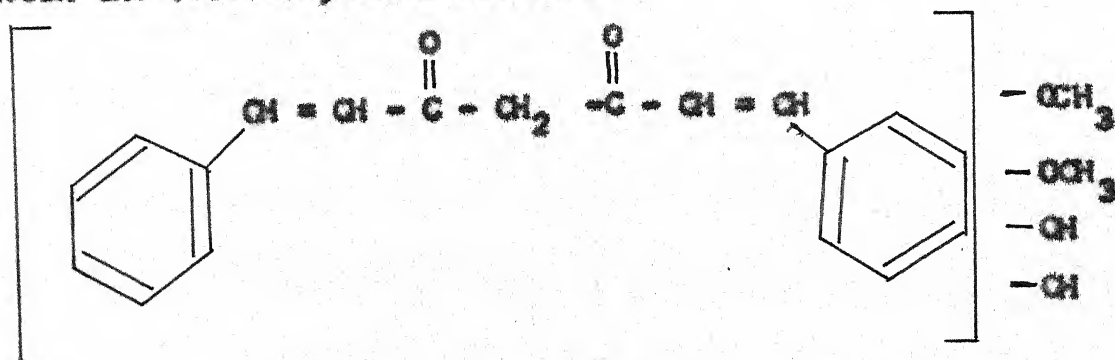
- (i) It gave red colour with ammonia.
- (ii) A spot of it ,on filter paper gave rose red colour with boric acid which changed to greenish blue when sprayed with ammonia.
- (iii) It gave a reddish brown colour with ethanolic ferric chloride.
- (iv) It gave an orange colour with lead acetate and zirconium oxy chloride.
- (v) It gave the red rose colour with sulphuric acid.
- (vi) It gave red colour with magnesium powder and hydrochloric acid.
- (vii) It gave a violet colour with sodium boro hydride.
- (viii) It gave red colour with sodium acetate.
- (ix) It decolourised alkaline potassium per manganate solution and reduced Fehling's solution.

The compound (F) , gave mass spectrum peak at  $M^+$  368. IR peaks 2950 and  $1740\text{ cm}^{-1}$  and UV absorption peaks at  $\lambda_{\text{max}}$  268 and 430 nm , showed that the compound (F) is a derivative of unsaturated phenol containing methoxyl and ketonic group. The presence of  $-\text{OCH}_3$  group was confirmed by Zeisel's estimation<sup>3</sup>.

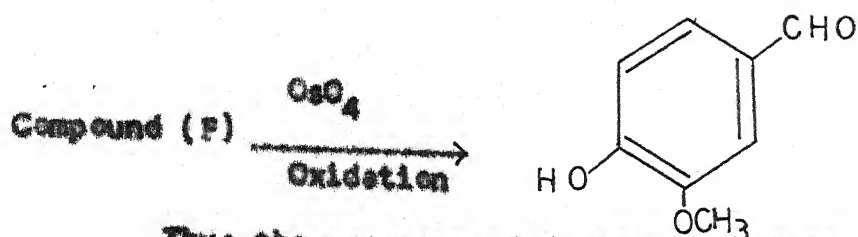
On acetylation with aqueous sodium hydroxide and acetic anhydride, the compound (F) , gave a yellow acetylated product, m.p.  $170^\circ\text{C}$  (IR peak,  $1760\text{ cm}^{-1}$ ,  $M^+$  452,  $\lambda_{\text{max}}$  295 and 402 nm. The estimation of acetylated product showed the presence of two acetyl groups.

On methylation with dimethyl sulphate the compound (F) gave , methylated product  $\text{C}_{23}\text{H}_{24}\text{O}_6$  . m.p.  $129-30^\circ\text{C}$  . (IR peak  $2940\text{ cm}^{-1}$  ,  $M^+$  396 ,  $\lambda_{\text{max}}$  262 and 420 nm ). It gave the presence of two free  $-\text{OCH}_3$  groups were estimated by Zeisel's method<sup>3</sup>.

From the above facts, the compound (F) should contain the following basic skeleton.

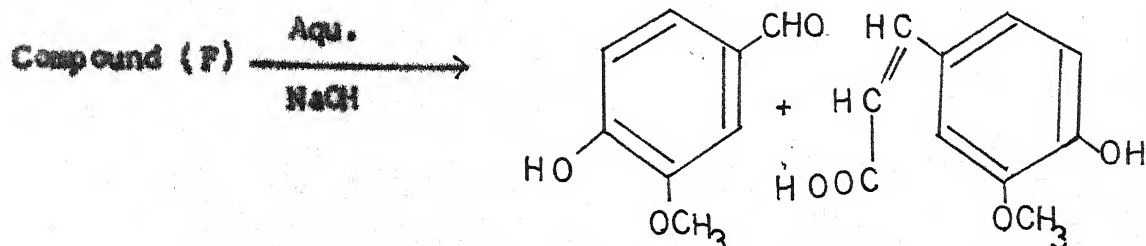


On oxidation with osmium tetroxide the compound (F) gave vanillin.



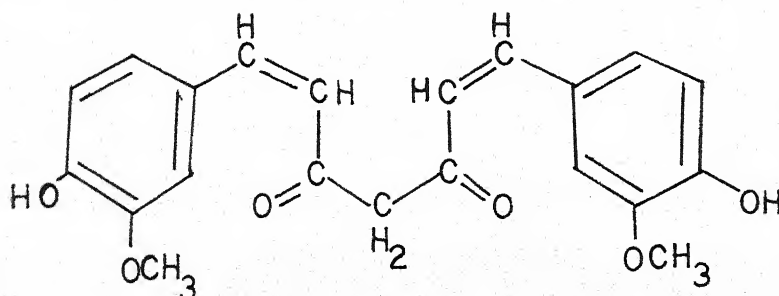
Thus the presence of Methoxy and Hydroxy groups in compound (F) was at 3,4-positions respectively to the benzene nucleus.

The compound (F) on degradation by aqueous alkali, gave vanillin and ferulic acid<sup>6</sup>, or 3-(4-hydroxy-3-methoxy phenyl)-2-propenoic acid.



It clearly shows that compound (F) resembles to Curcumin [1,7-bis(4-hydroxy-3-methoxy phenyl)-1,6-heptadiene-3,5-dione] .

On the basis of the above facts, the structure of compound (F) should be as follows :-



1,7-bis(4-hydroxy-3-methoxy phenyl)-1,6-heptadiene-3,5-dione  
or  
Curcumin



EXPERIMENTAL1. ISOLATION

The compound (F), having molecular formula  $C_{21}H_{20}O_6$ , m.p.  $194-95^{\circ}C$  was isolated with ethyl acetate from rhizome of *Curcuma longa* as described on page 86. It was recrystallised from methanol and the purity of the compound (F) was checked by Co-chromatography.

2. SOLUBILITY

The compound (F) was soluble in petroleum ether, benzene, ether, chloroform, ethyl acetate, acetone, methanol and ethanol.

3. THIN LAYER CHROMATOGRAPHY

The thin layer chromatoplates were prepared by silica gel-G and were activated at  $100^{\circ}C$  for one hour. The purity of the compound was checked by these plates using benzene : chloroform (1:1 v/v) as a solvent. Single spot was obtained when exposed with ammonia vapours.

4. ELEMENTAL ANALYSIS

Anal. data found (%)

C = 68.55

H = 5.4

M<sup>+</sup> 368

Calcd. for  $C_{21}H_{20}O_6$

C = 68.5 %

H = 5.45 %

M<sup>+</sup> 368

5. COLOUR REACTIONS OF COMPOUND (F)

The colour reactions of compound (F) was given on page 93 of the thesis.

## 6. ACETYLATION OF COMPOUND (F)

The compound (F) (50 mg) was acetylated with 10% aqueous sodium hydroxide (5 ml) and acetic anhydride (5 ml) in a 250 ml. flask. The reaction mixture was left at room temperature for 24 hours. The reaction mixture was poured in an ice cold water. A yellow precipitate was obtained and was washed well with cold water. It was then recrystallised from ethanol, m.p.  $171^{\circ}\text{C}$ . A diacetylated derivative of the compound (F) was obtained.

### 6.1. ELEMENTAL ANALYSIS OF ACETYL COMPOUND

The elemental analysis was done by Wisenberger<sup>4</sup> method as described by Belcher and Godbert<sup>5</sup>.

Anal. data found (%)	Calcd. for $\text{C}_{28}\text{H}_{24}\text{O}_8$
C = 66.7	C = 66.35 %
H = 5.55	H = 5.30 %
M <sup>+</sup> 452	M <sup>+</sup> 452

The molecular weight and molecular formula of the acetyl derivative, corresponds to the acetyl groups in the derivative.

## 7. METHYLATION OF THE COMPOUND (F)

The compound (F) (40 mg) was refluxed with benzene (100 ml.) and dimethyl sulphate (10 ml) over 1.0 gm anhydrous potassium carbonate with constant stirring for 48 hours on water bath. When the reaction was completed, it was cooled and filtered. After filtration



it was concentrated under vacuum. The concentrated solution was stirred with warm aqueous sodium bi carbonate. The mixture was extracted with chloroform. The extract was washed well, dried and evaporated. The residue was purified and recrystallised from ethanol, m.p. 128-30 °C.

[Found : C, 69.85, H, 6.45; calculated for  $C_{23}H_{24}O_6$  : C, 69.7, H, 6.05 %]. Molecular weight of the compound is 396.

#### 7.1 DETERMINATION OF METHOXYL GROUP (ZEISEL'S METHOD)

The determination of the methoxyl group in the compound (F) was carried out according to the well known Zeisel's method modified by Belcher, Fildes and Mutton.

The spiral scrubbing compartment of the apparatus filled with 3.5 to 4.0 ml of antimony potassium tartrate solution (10 %) in distilled water and the stopper was replaced. 10 ml of freshly prepared osmine bromine - sodium acetate solution in acetic acid (prepared by adding 0.3 ml of bromine to 10 ml of a 10% solution of sodium acetate in acetic acid) were added in the receiver which was connected to the scrubbing compartment.

The compound (F) (20 mg) accurately weighed in a small watch glass and allowed to slide down gently into the boiling flask. A drop of mercury was introduced followed by the addition of 5 ml of melted phenol (A.R.) and 5-6 drops of propionic anhydride were also added. The compound was dissolved in the solution mixture before the addition of 5 ml of hydrochloric acid. The connection were made gas tight.



Carbon dioxide gas was passed through the Kipp's Apparatus @ 60-70 bubbles per minute. The contents were heated and slowly brought to boil and the temperature adjusted so that the vapours did not rise more than half way to the condenser.

After heating for three hours the receiver and spiral inlet tube were removed from the washing compartment and with rinsed with distilled water. The contents of the receiver and washing were collected into a 250 ml Erlenmeyer flask containing 5 ml of 10% sodium acetate solution.

Ferric acid (A.R. grade) was added drop wise to the solution till the excess of bromine was destroyed, then 0.5gm of potassium iodide and 5 ml of 10% sulphuric acid were added. The contents were treated after five minutes with standard 0.05N sodium thiosulphate solution using starch as an indicator. A blank was also carried out simultaneously.

The percentage of methoxyl group was calculated by the amount of hypo consumed for titrated methyl iodide produced for each mole of the compound.

Anal.data found (%)

Calcd. for  $C_{23}H_{24}O_6$

Methoxyl group

Methoxyl group

percentage = 31.93

percentage = 31.31 %

### 9. HYDROGENATION OF COMPOUND (F)

The ethyl acetate solution of compound (F) was hydrolysed with 10% palladium-charcoal catalyst at  $100^{\circ}C$  and

50 atmospheric pressure for over night. The reaction mixture was cooled and filtered. The filtered solution was concentrated, cooled and extracted with chloroform. The evaporated extract gave a product which was crystallised from light petroleum ether (60-80°C) m.p. 95-96°C.

[Found : C, 67.65 ; H, 6.4 ; calculated for  $C_{21}H_{24}O_6$  : C, 67.75 ; H, 6.45 %].

Molecular weight of the compound (F) = 372.

#### 9. ALKALINE DEGRADATION OF COMPOUND (F)

The compound (F) (40 mg) was refluxed for two and half an hour with 5 % aqueous sodium hydroxide solution (33 ml). The mixture was distilled, when 15 ml of the distilled was obtained, it was collected and NaOH (500 mg) and added to the distillate in water and filtered. The filtrate was acidified with concentrated HCl and extracted with ethyl acetate and chloroform. It was dried, concentrated and chromatographed over silica gel -G. The two bands resolved were washed from silica with chloroform. The upper band gave vanillin (m.p. 80°C Lit. 81°C) and the lower band provided ferulic acid (m.p. 173°C, Lit. 174°C). The identity was confirmed by m.p.s., m.s.p.s. and super imposition of I.R. spectrum with authentic samples.

#### 10. OXIDATION OF COMPOUND (F)

The compound (F) (100 mg) in dioxan (15 ml) and water (1.5 ml) was treated with osmium tetra oxide (4 mg).



The reaction mixture was stirred (30 minutes) and added sodium periodate (750 mg) and after addition of sodium periodate, the reaction mixture was refluxed on water bath for forty eight hours. It was cooled and filtered. The filtrate solution was extracted with chloroform and solvent was evaporated. It was purified and recrystallised from ethyl acetate. It was identified with m.p., m.m.p. and co-chromatography with authentic sample.

# 11. ABSORPTION SPECTRAL DATA OF COMPOUND (F)

## (1) U.V. SPECTRAL DATA (nm)

Compound (F)  $\lambda_{\max}$  (CH<sub>3</sub>OH) ; 268 (4.09) and 430 nm (4.14).

Acetylated product  $\lambda_{\max}$  (C H<sub>3</sub>CH) 255 (4.10) and 402 nm (4.60).

Methylated product  $\lambda_{\max}$  (CH<sub>3</sub>OH) 262 (4.08) and 420 nm (4.66).

## (11) I.R. SPECTRAL DATA (KBr)

The prominent peaks in I.R. spectrum of the compound (F) and derivatives were :

Compound (F) 2850, 1740 cm<sup>-1</sup>

Acetylated product 1760 cm<sup>-1</sup>

Methylated product 1690 and 1667 cm<sup>-1</sup>

## (111) <sup>1</sup>H-N.M.R. SPECTRAL DATA (CDCl<sub>3</sub>)

$\tau$ (ppm)	No. of H	Assignment
Compound (F) -		
2.42, d	2 H, J=16Hz	4,4' - H <sub>2</sub>
2.70, d	2 H, J= 2Hz	6,6' - H <sub>2</sub>



$\tau$ (ppm)	No. of H	Assignment
2.84, dd	2H, J=2 and 8Hz	10,10' -H <sub>2</sub>
3.14, d	2H, J= 8Hz	9,9' - H <sub>2</sub>
3.34, d	2H, J=16Hz	3,3' - H <sub>2</sub>
4.04, s	1H	1-H
6.09, s	6H	2XOMe
Acetylated product		
2.34, d	2H, J=16Hz	4,4' - H <sub>2</sub>
2.6-3.1, m	6H	AriH
3.44, d	2H, J=16Hz	3,3' - H <sub>2</sub>
4.17, s	1H	1-H
6.14, s	6H	2XOMe
7.70, s	6H	2XCOMe
Methylated product		
2.42, d	2H, J=16Hz	4,4' -H <sub>2</sub>
2.88, dd	2H, J=2.8Hz	10,10' -H <sub>2</sub>
2.94, d	2H, J=2Hz	6,6' - H <sub>2</sub>
3.16, d	2H, J =8Hz	9,9' - H <sub>2</sub>
3.54, d	2H, J=16Hz	3,3' - H <sub>2</sub>
4.20, s	1H	1-H
6.10, s	12H	4X OMe

$\tau$ (ppm)	No. of H	Assignment
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## Hydrogenated product

3.19, d	2H, $J=8\text{Hz}$	$10,10' - \text{H}_2$
3.34, s	2H	$6,6' - \text{H}_2$
3.39, d	2H, $J=8\text{Hz}$	$9,9' - \text{H}_2$
4.46	2H	ArOH
4.60, s	1H	1 - H
6.17, s	6H	2XOMe
7.0-7.6 m	8H	-

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## CHAPTER - IV

### CHEMICAL EXAMINATION OF BARK OF *FICUS GLOMERATA*



The plant *Ficus glomerata*, commonly known as Gular or Umar, belongs to the family Moraceae. It is a common tree found in all over tropical region of the country.

The tree is about 60 feet high. The bark is smooth, reddish brown in colour and young shoot is glabrous pubescent or scaberulous. Leaves are membranous, long ovate in shape, when mature, usually some what pubescent beneath, base rounded or acute. The male, female and gall flowers are all found together in the same receptacles. It is leafless during the rainy season and the figs ripen between April and July. The plant *Ficus glomerata* is a medicinal plant used in indigenous system of medicine.<sup>1,2</sup>

The bark, leaves and fruits are used in native medicine.

The powder of the leaves are given in bilious disorders, menorrhagia and haemoptysis. It is useful for mouth washing in spongy gums.

The bark is important for medicine. It is used haematuria, haemoptysis, menorrhagia, diabetes and dysentery. Its paste with sweet oil is applied over ulcers.

The latex is used in mumps and other glandular swellings, rheumatic joints and pains in the chest.

The figs are an efficacious remedy in diarrhoea during pregnancy. A decoction of the dried figs is

an excellent mouth wash for sore throat and aphthous complaints of the mouth. The fruits have been used in the indigenous system of medicine since ancient times.<sup>3,4</sup>

CONSTITUENTS	PART OF THE PLANT	REFERENCES
(1) $\beta$ -sitosterol, lup-eol acetate, $13\alpha$ , $14\beta$ , $17\beta(H)$ $20\alpha(H)$ -lanosta-8,22-diene-3 $\beta$ -ol	Fruits	Indian J. Chem., Sec. B. <u>17B</u> (1), 37-8, (1979) (Eng.)
(11) Glucan acetate, $\beta$ -Amyrin, $\beta$ -sitosterol	Leaves	J. Indian Chem. Soc. <u>48</u> (12), 1165-9, (1971) (Eng.)
(11 <sub>1</sub> ) Long Chain polyphenols	Leaves	Acta, Bio chem. Pol, <u>20</u> (4), 343-50, (1973) (Eng.).

The literature survey reveals that no work has been done on the bark of the plant *Ficus glomerata* separately. An attempt has been made in this chapter to study thoroughly for the active chemical constituents from the bark of the plant.



## EXTRACTION AND ISOLATION OF CHEMICAL CONSTITUENTS OF THE BARK OF FICUS GLOMERATA

The bark of the plant *Ficus glomerata* was collected locally and identified for their authenticity in the Botany Department of D.V. (P.G.) College, ORAI.

The dried and crushed bark was used for extraction.

### EXTRACTION OF THE BARK.

2.0 kg. dried and crushed bark was defatted with petroleum ether (60-80°C) in Soxhlet extractor for 36 hours. The defatted material was extracted with benzene and ethyl acetate. The ethyl acetate extract of the bark was collected and concentrated under reduced pressure to give a semi solid mass. It was chromatographed by silica gel-G column, using benzene : chloroform (1 : 1 v/v) as an eluent. The eluted solvent, on concentration gave a solid compound (G). The purity of the compound was checked by thin layer chromatography using silica gel-G as adsorbent and n-butanol : acetic acid : water (4 : 1 : 5 v/v) as an eluent. The spot was detected by ~~iodine chamber~~ putting the plate in iodine chamber.

The residue was refluxed with absolute ethanol (95%) in several lots, a dark brown coloured extract was obtained which was concentrated to a viscous mass under reduced pressure. This extract was refluxed with petroleum ether, benzene and ethyl acetate

in an order but no fruitful results were obtained with it.

The remaining residue was separated by column chromatography using alumina as adsorbent, the first fraction on elution with pet. ether; benzene (1;1 : v/v) gave compound (H) and second fraction was eluted with benzene : chloroform (2:1 v/v) afforded compound (I) . Both compound (H & I) were recrystallised from methanol.

## BARK OF FICUS GLOMERATA

Defatted with Pet. ether  
(60-80°C) in a Soxhlet  
extractor for 24 hours.

Extract

Defatted material

Refluxed with  
ethyl acetate

Extract

Chromatographed over  
silica gel-G column  
and eluted with benzene:  
chloroform (1:1 v/v).

A white crystalline solid

Compound (G)

Residue

Refluxed with  
Ethyl acetate  
and chromatographed over  
Alumina column

Eluted with Benzene:  
Pet. ether (60-  
80°C) (1:1 v/v)

White crystalline  
needles

(Compound (H))

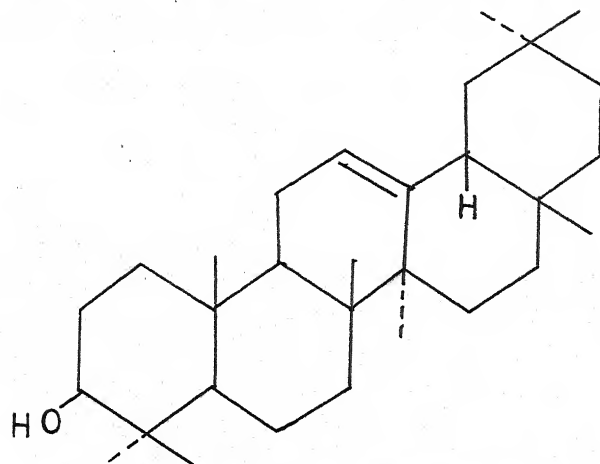
Eluted with  
Benzene : Ethyl  
acetate (1:1 v/v).

Compound (I)



CHEMICAL STUDY OF COMPOUND (G)

Since the compound (G) showed all the characteristic colour reaction, degradative studies and spectral measurement similar to the aglycone of the compound (C). Thus all the reaction and tests have been already described on page from 43 to 47 . Therefore, the structure of the compound (G) was as follows:-



Olean-12-en-3-β-ol

(β - Anysrin)

### CHEMICAL STUDY OF COMPOUND (H)

A white crystalline compound (H) having molecular formula  $C_{32}H_{52}O_2$ .  $M^+$  468, m.p.  $180^\circ C$ ,  $[\alpha]_D^{23} \pm 0$ , isolated with the bark of *Ficus glomerata* as described on page 107. It was recrystallised from methanol : Chloroform (1:1 v/v). It was soluble in benzene, ethyl acetate, chloroform, acetone, methanol and ethanol.

The compound (H) gave the following colour reactions:

- (i) It gave positive Liebermann Burchard Reaction<sup>5</sup>.
- (ii) It gave positive Moller's test<sup>6</sup>.
- (iii) It gave Selkowaski reaction<sup>7</sup>.
- (iv) It decolourised potassium permanganate solution and bromine water.
- (v) It gave positive Ruzicka reaction<sup>8</sup>.

From the foregoing colour reactions it was obvious that the compound (H) was a triterpene and not a steroid.

The compound (H), having  $M^+$  468, 1 R peaks at  $1372, 1362\text{ cm}^{-1}$  (gem dimethyl function),  $1451\text{ cm}^{-1}$  (methylene group) confirmed the above aspect.

The compound (H) in U V spectrum did not give absorption peaks<sup>9,10,11</sup> above 212 nm, showing the

absence of conjugated system of double bonds. The IR peaks at 1724 and 1244  $\text{cm}^{-1}$  indicated the presence of acyl group in the compound (H).

The hydrolysis of compound (H), with 5% alcoholic sodium hydroxide, gave another compound ( $\text{H}_1$ ), m.p. 174  $^{\circ}\text{C}$ ,  $[\alpha]_{\text{D}}^{21} + 2^{\circ}$ ; molecular formula  $\text{C}_{30}\text{H}_{50}\text{O}$ , IR peaks at 3395, 1035  $\text{cm}^{-1}$ ,  $\text{M}^+$  426 which on acetylation with acetic anhydride and pyridine gave original compound (H). It clearly indicated that the compound (H) is an acetylated derivative of tetracyclic triterpene.

Compound (H), in NMR spectrum gave 52H atoms in the molecule, 24 H atoms as methyl group. The existence of 8 methyl groups in a  $\text{C}_{32}$  molecule with side chain at  $\text{C}_{17}$  (by mass spectrum) and oxygen as acetoxy group, leaves no doubt about acetyl derivative of a tetracyclic triterpene.

The positions of  $\text{C}_{18}$  and  $\text{C}_{19}$  methyl groups were confirmed by NMR peaks at  $\tau$  9.20 and  $\tau$  9.14 respectively. The signal at  $\tau$  5.0 showed the presence of two ethylenic protons. This signal seemed as an unresolved triplet indicating that the presence of  $-\text{CH}_2-$  group. The signal at  $\tau$  7.93 showed the presence of three protons of acetyl methyl group.

The mass spectrum of compound (H) showed molecular ion peak at  $m/e$  468 and peaks at  $m/e$  425, 453 and 357 corresponded to the loss of prop-yl,

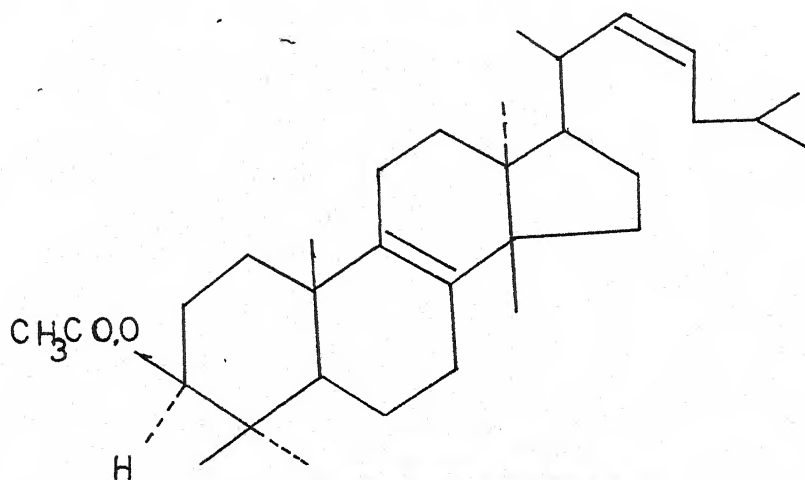


methyl and 6-methyl heptenyl ( $C_8H_{15}$ ). These fragments indicated that there must be a double bond at  $\Delta^{22(23)}$ . The other peaks at  $m/e$  317, 315 and 289 are due to loss of  $C_{11}H_{19}$  (151),  $C_{11}H_{21}$  (153) and  $C_{13}H_{23}$  (179).

The compound ( $H_1$ ) on mass spectrum has a molecular ion peak at  $m/e$  426 which confirmed the presence of one acetyl group in original compound (H). The peak at  $m/e$  383 is due to loss of prop-yl from the side chain and  $m/e$  315 due to loss of complete side chain 6-methyl heptenyl ( $C_8H_{15}$ ). This is further showed that one double bond is present in side chain and its position is  $\Delta^{22(23)}$ . The peak at  $m/e$  275 is due to loss of fragment  $C_{11}H_{19}$ . The other peaks at 273, 247, 207 are due to loss of  $C_{11}H_{21}$ ,  $C_{13}H_{23}$  and  $C_{16}H_{27}$  respectively. The position of second double bond indicated at position 8 and 9 due to the presence of peaks at  $m/e$  247 and  $m/e$  239 in compound (H) and ( $H_1$ ) both.

Compound ( $H_1$ ) when treated with Jones reagent<sup>12</sup> in acetone at room temperature gave a ketone (compound  $H_2$ ) molecular formula  $C_{30}H_{48}O$ ,  $M^+$  424, m.p.  $178^\circ$   $[\alpha]_D^{25} + 4.5^\circ$ . It had no UV maxima at 212 nm indicated the absence of conjugated keto system. Its IR peaks at  $1706\text{ cm}^{-1}$  indicated the presence of carbonyl group in a six membered ring.

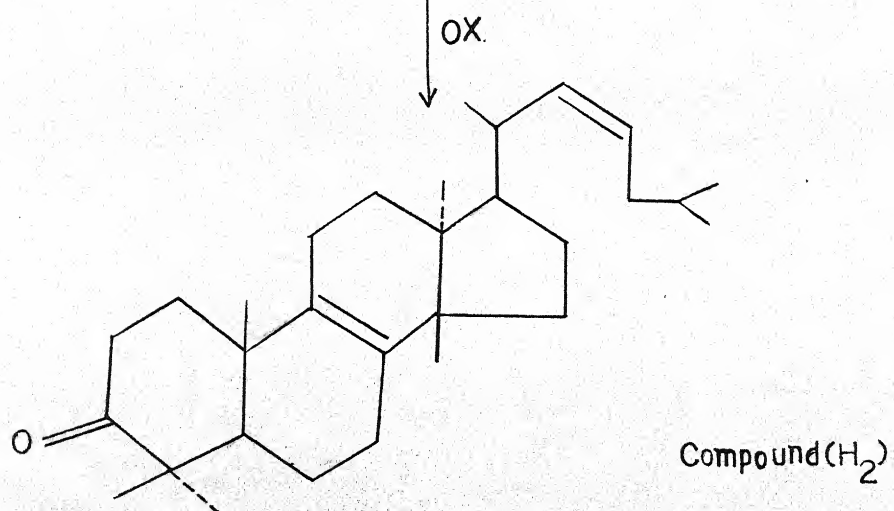
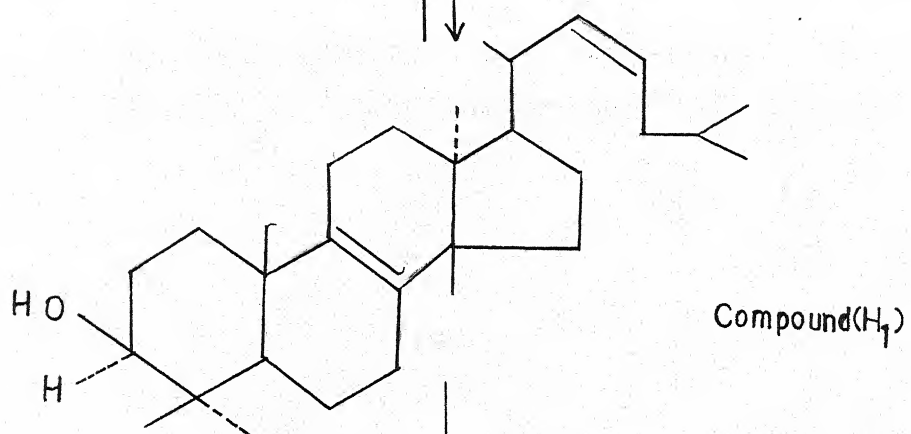
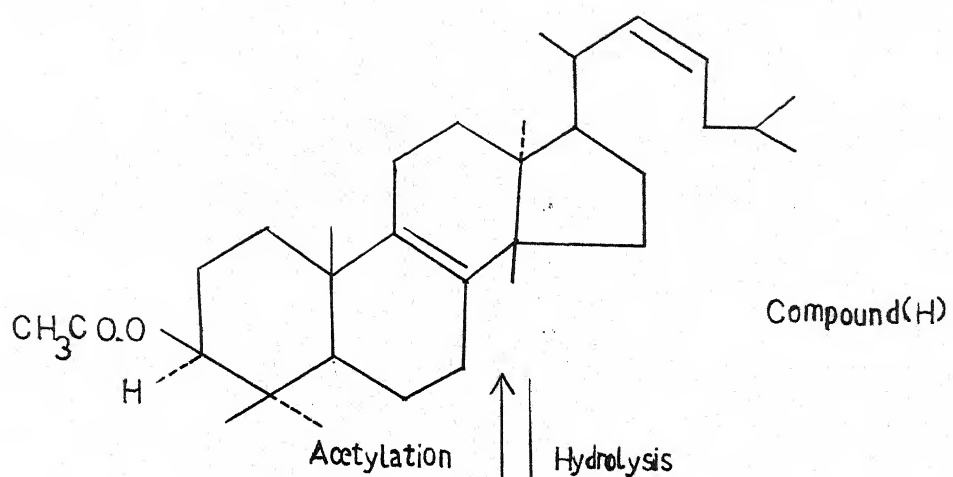
The compound (H) on hydrogenation with Adam's reagent<sup>13</sup> in ethyl acetate gave a dihydro compound, molecular formula  $C_{32}H_{54}O_2$ ,  $M^+$  470, m.p.  $145^\circ C$ ,  $[\alpha]_D^{23} - 5^\circ$ . This dihydro compound was found similar to tirucallinol acetate(  $13\alpha$ ,  $14\beta$ ,  $17\beta$  (H),  $20\alpha$  (H) lanosta-8-en-3 $\beta$ -acetate) by m.p., m.m.p. and superimposition of IR spectrum. Thus the compound (H) is a gluanol acetate  $13\alpha$ ,  $14\beta$ ,  $17\beta$  (H),  $20\alpha$  (H) lanosta-8, 22-diene-3 $\beta$ -acetate.



Gluanol acetate.

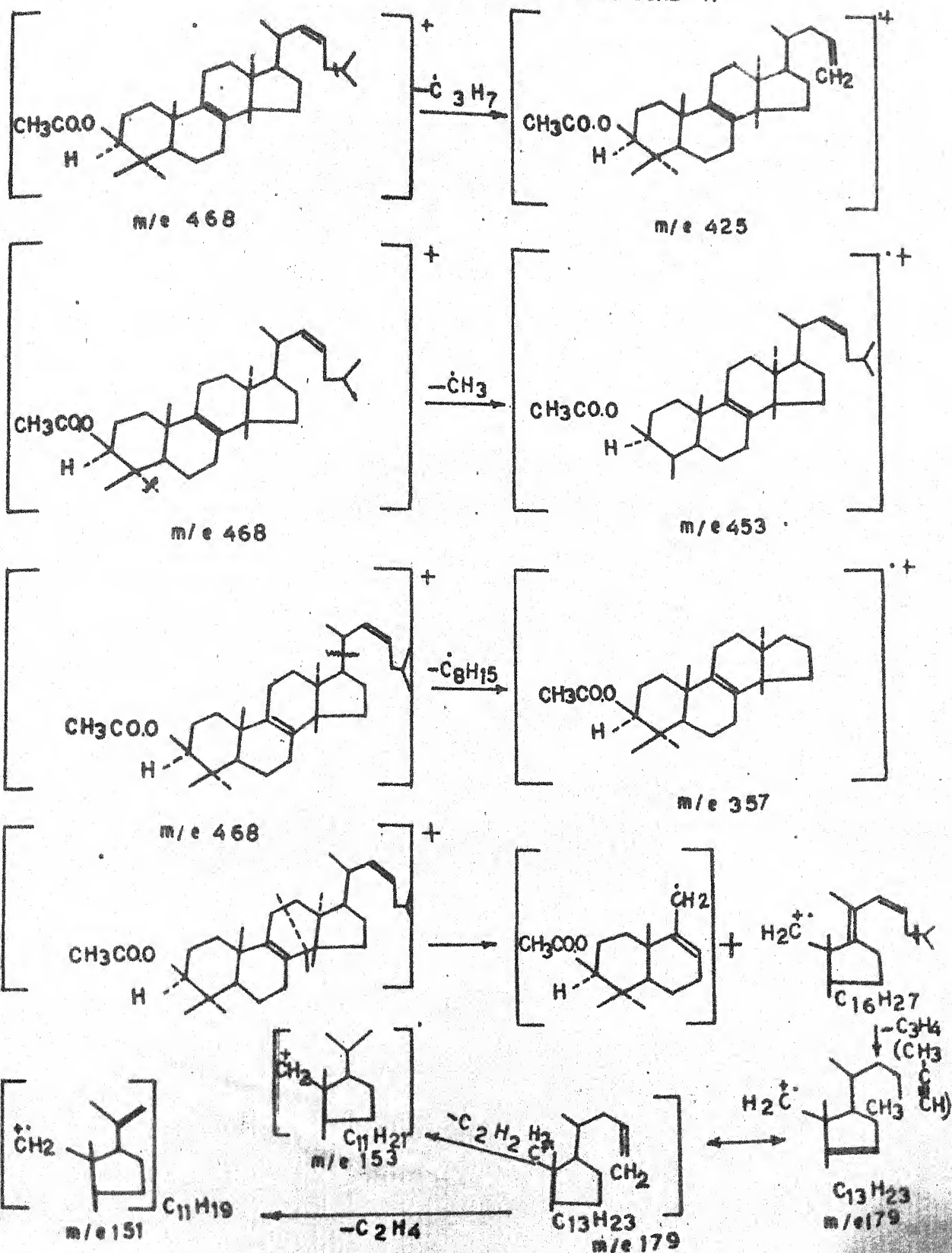
$13\alpha$ ,  $14\beta$ ,  $17\beta$  (H),  $20\alpha$  (H) lanosta-8, 22-diene-3 $\beta$ -acetate.

The chemical reactions involved during the determination of structure of compound (H) as follows.





# MASS FRAGMENTATION PATTERN OF COMPOUND . H



EXPERIMENTALISOLATION AND PURIFICATION

The compound (H) was isolated with ethanol from the bark of the plant *Ficus glomerata* as described on page 107 . It was recrystallised from methanol : chloroform (1 : 1 v/v) into white crystalline needles, m.p.  $180^{\circ}\text{C}$  ,  $[\alpha]_{\text{D}}^{25} \pm 0^{\circ}$ . The compound (H) was soluble in all organic solvents. Its purity was checked by co-chromatography with methanol : ethyl acetate (1 : 1 v/v) mixture.

2. COLOUR REACTIONS OF THE COMPOUND (H)-

The colour reactions of the compound (H) are given below.

(i) Liebermann Burchard Reaction<sup>5</sup> -

The compound (H) (5 mg) dissolved in chloroform (2 ml) added , few drops of acetic anhydride and concentrated sulphuric acid, gave red colour.

(ii) Noller's Test <sup>6</sup>.

On addition of few drops of thionyl chloride in the compound (H) , gave a deep red colour (Thionyl chloride solution was prepared by adding 0.01% stannic chloride in pure thionyl chloride).

(iii) Salkowski Reaction<sup>7</sup>.

On treatment with sulphuric acid, the chloroform solution of the compound (H) gave a yellow colour which changed into deep red.

(iv) The compound (H) decolourised aqueous potassium permanganate solution and bromine water.

(v) Ruzicka Reaction<sup>8</sup>.

The compound (H) dissolved in chloroform (2 ml) and added the mixture of tetranitromethane and chloroform (1:1 v/v), a yellow colour was obtained.

(vi) The ethanolic solution of the compound (H) with ethanolic solution of the digitonin<sup>14</sup>, gave a white precipitate.

3. ELEMENTAL ANALYSIS .

Anal. data found (%)

C = 80.90

H = 11.07

Mol. wt. = 456

(By mass spectrum)

Calcd. for  $C_{32}H_{52}O_2$

C = 81.99 %

H = 11.18 %

Mol. Wt. = 454

4. HYDROLYSIS OF THE COMPOUND (H).

Compound (H) (50 mg) was refluxed with 5 % ethanolic sodium hydroxide (12 ml) for 6 hours. The reaction mixture was cooled, concentrated and poured in ice cold water. It was shaken with solvent ether. The ethereal layer was washed with distilled water to remove the alkali and on concentration under reduced pressure to give a solid mass which was recrystallised from methanol : chloroform (1:1 v/v) to give a white crystalline



compound named ( $H_1$ ), having molecular formula  $C_{30}H_{50}O$ .  
 m.p.  $174^{\circ}C$ ,  $[\alpha]_D^{21} + 2^{\circ}$ .

#### 4.1 - ELEMENTAL ANALYSIS OF COMPOUND ( $H_1$ ).

Anal. data found(%)	Calcd. for $C_{30}H_{50}O$ .
C = 84.38	C = 84.51 %
H = 12.08	H = 11.74 %
Mol. Wt. = 426	Mol. Wt. 426

#### 5. ACETYLATION OF COMPOUND ( $H_1$ ).

Compound ( $H_1$ ) (50 mg) was acetylated with 10 ml of acetic anhydride and pyridine (10 ml). The reaction mixture was refluxed on water bath for 10 hours. On complete acetylation, the reaction mixture was cooled and poured into an ice cold water. A yellow precipitate was obtained which was filtered and washed well with distilled water. After drying it was recrystallised from methanol : chloroform (1:1 v/v) into colourless needles of acetyl derivative, m.p.  $150^{\circ}C$ , molecular formula  $C_{32}H_{52}O_2$ ,  $[\alpha]_D^{25} + 0^{\circ}$ .

#### 5.1 ELEMENTAL ANALYSIS OF COMPOUND ( $H_1$ )

Anal. data found(%)	Calculated for $C_{32}H_{52}O_2$
C = 81.65	C = 81.99 %
H = 11.00	H = 11.18 %

#### 6. OXIDATION OF COMPOUND ( $H_1$ ).

The compound ( $H_1$ ) (30 mg) was dissolved in dry acetone and at  $20^{\circ}C$  the Jones's Reagent

(8 N chromic acid) was added dropwise until a permanent orange colour appeared. The reaction mixture was kept for 10 minutes and the diluted with distilled water and shaken with ether. The ether layer was separated out and washed with 5% sodium bi carbonate solution and then distilled water. The ether was evaporated. After evaporation the residue was left which was recrystallised from methanol : acetone (2:1 v/v), m.p.  $174^{\circ}\text{C}$ ,  $[\alpha]_{\text{D}}^{25} + 4.5^{\circ}$  molecular formula  $\text{C}_{30}\text{H}_{48}\text{O}$ .

It gave positive Zimmerman test<sup>15</sup> which confirmed the ketonic group at position- 3.

#### 6.1 ELEMENTAL ANALYSIS OF COMPOUND ( $\text{H}_1$ ).

Anal. data found(%)	Calcd. for $\text{C}_{30}\text{H}_{48}\text{O}$
C = 84.52	C = 84.5%
H = 11.79	H = 11.70 %

#### 7. HYDROGENATION OF COMPOUND (H).

In the presence of Adam's catalyst (Adam's Platinum oxide) (20 mg) in ethyl acetate (50 ml), the compound (H) (50 mg) was hydrogenated in the atmosphere of hydrogen for 10 hours. The solution was filtered and distilled excess of ethyl acetate. The remaining residue was crystallised from methanol : chloroform to a white crystalline solid i.e. dihydro- compound of the compound (H). It was found similar to the tirucallenol acetate, (  $13\alpha$ ,  $14\beta$ ,  $17\beta$  (H),  $20\alpha$  (H) Lanota-8- en-  $3\beta$  -acetate). It was confirmed by m.p., m.m.p., co-chromatography and super-imposition of I R spectrum with authentic sample.

M.P.  $145^{\circ}\text{C}$ ,  $[\alpha]_{\text{D}}^{23} -5^{\circ}$ , molecular formula  $\text{C}_{32}\text{H}_{54}\text{O}_2$ ,  
 $+458$ .

### 7.1 ELEMENTAL ANALYSIS

Anal. data found(%)

Calcd. for  $\text{C}_{32}\text{H}_{54}\text{O}_2$

C = 81.63

C = 81.70 %

H = 11.35

H = 11.48 %

### 8. ABSORPTION SPECTRAL DATA OF COMPOUND (H)

#### 8.1 I R SPECTRAL DATA (KBr) ( $\text{cm}^{-1}$ )

The prominent I R peaks were at-  
 1035, 1244, 1362, 1372, 1451, 1724 and  $3395\text{ cm}^{-1}$ .

#### 8.2 - N M R SPECTRAL DATA ( $\text{CDCl}_3$ ) .

The  $\tau$  values of compound (H) are  
 given as follows:

5.0, 7.93, 9.14 and 9.20

#### 8.3- MASS SPECTRAL DATA

The important m/e peaks of the compound  
 (H) are given as follows.

468, 453, 425, 383, 357, 315, 289, 275, 273, 247,  
 207, 179, 153 and 151.



# CHEMICAL STUDY OF COMPOUND (I)

A white crystalline solid compound (I) having m.p.  $211-12^{\circ}\text{C}$  ,  $[\alpha]_{\text{D}}^{25} + 27.9$ , molecular formula  $\text{C}_{30}\text{H}_{50}\text{O}$  was isolated with ethanol from the bark of *Ficus glomerata* as described on page 107. The compound (I) was recrystallised from ethanol and its homogeneity was checked on chromatoplates of silica gel G using chloroform : benzene (2 : 1 v/v) as an irrigating solvent and the spot was revealed by chlorosulphonic reagent. It was soluble in ethyl acetate , acetone , chloroform , methanol , ethanol and pyridine. Its molecular formula was further supported by the molecular ion peak at m/e 426 in the mass spectrum.

The compound (I) gave the following colour reactions :

- (i) It gave a deep red colour in Liebermann Burchard reaction<sup>5</sup>.
- (ii) It gave a deep red colour in Moller's test<sup>6</sup>.
- (iii) It gave a yellow colour changing to red with Salkowski reagent<sup>7</sup>.
- (iv) It developed a red colour with greenish fluorescence , when chloroform solution was boiled with an excess of acetyl chloride and a little of zinc chloride (Tschugajew Reaction<sup>16</sup> ) .
- (v) It gave a reddish violet colour in tetra nitro methane (Razicka test<sup>8</sup>).

(vi) It gave a reddish violet colour in the Brieskorne test.<sup>17</sup>

(vii) It did not give precipitate with digitonin reagent.<sup>14</sup>

From the above colour reactions it is clear that the compound (I) has a triterpenoid nucleus. These reactions are specific according to Steiner and Holtzner.<sup>18</sup> The reddish violet colour in Brieskorne test<sup>17</sup> and no precipitate with digitonin<sup>14</sup> confirmed the absence of steroid compound.

From the molecular formula and colour reaction it is evident that the compound (I) is a triterpenoid.

It does not belong to the  $\alpha$ - or  $\beta$ -amyrin series of pentacyclic triterpenes, which is shown by the following facts.

(i) There was no typical UV absorption which showed the double bonds at position 12 and 13 which belongs to the  $\alpha$ - or  $\beta$ -amyrin series (Halsall<sup>19</sup>).

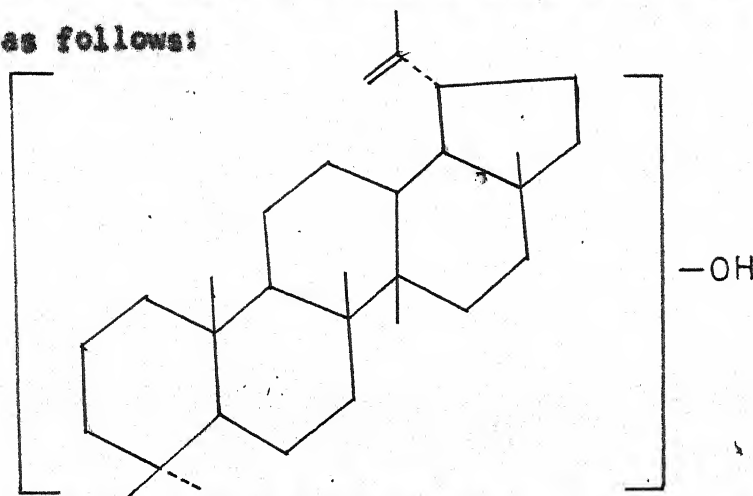
(ii) The absence of IR absorption peak at 1650-1667  $\text{cm}^{-1}$ , 3023-3030  $\text{cm}^{-1}$  and 904, 918, 928  $\text{cm}^{-1}$  also obstructed the presence of ethylenic bond at the position-12-13 in the pentacyclic triterpenes.

The compound (I) belongs to the lupeol series of triterpenic compound which is shown by the following facts.

The IR absorption peaks at 1699 and 885  $\text{cm}^{-1}$  are the characteristic peaks of the ethylenic double bond between position 20 : 29 in pentacyclic triterpene group of lupeol series.

It showed the absence of ketones, lactones or carboxylic acids by testing usual tests.

On acetylation with acetic anhydride and pyridine mono acetate derivative, m.p.  $213-14^{\circ}\text{C}$  molecular formula  $\text{C}_{32}\text{H}_{52}\text{O}_2$ ,  $M^+ 468$   $[\alpha]_D^{25} + 47^{\circ}$  (in chloroform) and on benzylation with benzoyl chloride, a mono benzoate derivative, m.p.  $269-70^{\circ}\text{C}$ ,  $[\alpha]_D^{25} + 61^{\circ}$  (in chloroform), molecular formula  $\text{C}_{36}\text{H}_{54}\text{O}$ ,  $M^+ 502$ , were obtained which confirmed the presence of one hydroxyl group in the compound (I). It was further confirmed by the I R spectrum in Nujol at  $3320\text{ cm}^{-1}$  ( $-\text{OH}$  group). Thus the structure of the compound (I) may be as follows:

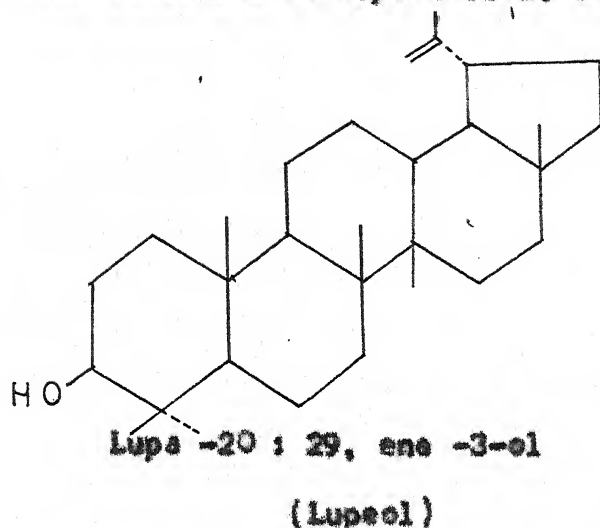


Compound I, on chromic acid oxidation gave a product which responded for the test of 3-keto group (Zimmermann colour test)<sup>15,19</sup>. Hence it has a secondary hydroxyl group and situated at position - 3.

The above structure was further supported by the absorption peaks at  $3325$  and  $1110\text{ cm}^{-1}$  in the I R spectrum and a quartet between  $6.9 - 6.65$  in the N M R spectrum<sup>20</sup>.



Thus the compound (I) is a Lupa-20 : 29, ene, 3-ol (Lupeol). The structure of lupeol is as follows:-



The compound (I) was further confirmed by the following observations -

(i) Mixed melting point, optical rotation and NMR spectrum of the compound (I) and its acetate derivative agreed well with those of lupeol acetate<sup>21</sup>.

(ii) The IR spectrum  $\lambda_{\text{KBr Max}}$  ( $\text{cm}^{-1}$ ) 163 and  $895 \text{ cm}^{-1}$  ( $\text{C}=\text{CH}_2$ ) and NMR spectrum  $\text{CDCl}_3$ , 60 mc, signals at 5.30 - 5.42 (2H;  $\text{HC}=\text{CH}_2$ ) and a sharp singlet at 8.3 (3H,  $\text{C}-\text{CH}_3$ ) confirmed the presence of isopropenyl group in the compound (I).

(iii) The mass fragmentation spectrum of the compound (I) confirmed the Lupene derivative. The prominent peaks are at  $m/e$  426 (molecular ion), 411 ( $\text{M}-\text{CH}_3$ ), 393 ( $\text{M}-\text{CH}_3 - \text{H}_2\text{O}$ ), 383 ( $\text{M}-\text{C}_3\text{H}$ )  $m$  370, 315, 220, 218, 207, 205, 191, 189 and 187.

According to Djerassi<sup>22</sup> the loss of 43 mass unit  $C_3H_7$  is pronounced in certain members of the lupane group but becomes minimum in the presence of isopropenyl function. They exhibit loss of methyl group (M-15 units). The most abundant fragment occurred at  $m/e$  205 and was observed in the spectra of all pentacyclic triterpenoids. Other important peaks were at  $m/e$  191 and 189.

The UV and NMR studies of the compound (I) proved the  $\beta$  -orientation of the hydroxyl group (equatorial). The NMR spectrum of the acetate of the compound (I) showed signals at 4.32 to 5.00 region of the spectrum which is characteristic of the proton  $\alpha$  -to the secondary equatorial hydroxy acetates<sup>23</sup>. The IR spectrum of the compound (I) as well as of its acetate did not show any complex band in  $1220-1260\text{ cm}^{-1}$  of the IR spectra, characteristic of the presence of axial hydroxyl group at position -3 in triterpenoids<sup>24</sup>. The acetate of the compound (I) could be easily hydrolysed than the benzoate in alkaline hydrolysis showing the presence of equatorial hydroxyl group at position -3 in the triterpenoid<sup>25</sup>.

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